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Encyclopedia of Medical Immunology

Autoimmune Diseases

Springer Reference

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With 227 Figures and 121 Tables

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Introduction

The concept of an encyclopedia derives from the Greek words for gathering together or "encircling" knowledge and learning. Indeed, Diderot and the French encyclopedists of the mid 18th century aimed to bring together all of the world's knowledge in one giant publication. Our ambitions today are more modest but "encapsulating" existing knowledge of a defined topic is still a reasonable basis for decision making in the present and planning for the future. The Encyclopedia of Medical Immunology follows in the encyclopedist tradition. At the present time, however, progress is proceeding at such a rapid pace that a static volume, no matter how extensive, could never do justice to this dynamic subject. Thus our present encyclopedia is based on the concept that articles will be linked to current research and updated on a regular basis. The reader needs to gain an understanding of medical immunology not only at the date of publication, but on a continuing basis.

The immune system, as a vital component of normal physiology, participates in establishing and maintaining the well-being of the host. Its core responsibility is to prevent or control infection and malignancy. Immune functions can be divided into constituative and adaptive. Inherited innate immunity takes its origins from most primitive cellular functions of recognition and nutrition. In animals, it evolved through invertebrates as a group of formed barriers and a system of cells and cell products for promptly dealing with harmful invaders or preventing clonal amplification of malignant cells. In vertebrates, in addition to innate immunity, an adaptive immune system provides a more focused and potent response, but one that requires more time to mobilize. It utilizes a novel system of hypermutation and recombination to provide a sufficiently broad repertory of receptors to recognize and eliminate, in principle, any potential microbial invader. In establishing and maintaining such a wide repertory of recognition structures, the adaptive immune system inevitably recognizes many epitopes on molecules within the body of the host. Thus, the same protective effector mechanisms of the healthy immune system, if out of control, can produce harm in the form of the immune mediated disorders described in these Volumes.

The most frequent disorders of the immune system are deficiencies. If the immune system fails to perform its core function of protection, infectious or malignant disease can follow. Most of these immune failures result from germ line inheritance of mutations in genes regulating the innate or adaptive

immune systems. The most frequent sign of an immune deficiency disease is infection due to one or more of the myriad microorganisms that inhabit the human environment.

A second group of immune-related illnesses results from loss of normal immunologic homeostasis. The regulatory devices that normally limit immune responses are inadequate. The failure may result from deficiencies, either inherited or acquired, of the overall regulatory machinery. Rather than a decrease in homeostatic regulation, immune disease can result from augmented immune responses. Powerful adjuvants, providing the non-antigen-specific signals, may overcome even normally functioning immune regulation.

Both types of immune-mediated disease are considered in our encapsulated knowledge. Allergies result from exposures to foreign substances that are harmless in the majority of individuals. As a group, allergic diseases affect at least 10% of the population and appear to be increasing over time in many populations. In contrast to an exaggerated response to foreign antigens, autoimmunity is the consequence of the "forbidden" recognition of some antigens in the host's body. Like allergic disease, autoimmune disease represents an uncontrolled immune response. Because allergic and autoimmune diseases can occur in different organ systems in the body, they can differ greatly in their clinical presentation, even though they share many genetic and regulatory features.

The goal in all medical immunology is to alleviate or prevent illness. If a disease is related to an inadequate immune response or to an overwhelming challenge, an intervention in the form of vaccination is a historically proven approach. Preventive vaccinations may be the most successful public health measure of the 20th century. New vaccines directed to oncoming newly emerging infectants or subtypes remains a major goal of current immunologic research. Potential adverse effects of vaccines also require constant attention. These days vaccines are being tested as a way of limiting or reducing malignant tumors.

Immunotherapy is a more modern success story as biological agents such as monoclonal antibodies and receptor-blocking ligands are increasingly available for control of diseases due to immunological derangement.

The need for an Encyclopedia of Medical immunology is compelling. Our encyclopedia is divided for convenience into the four subject areas discussed above: Immune deficiency diseases, allergic diseases, autoimmune diseases and vaccines. Each of these areas has significant and immediate relevance to medical practice and public health. Each is a growing area of research.

By bringing together these different areas in one comprehensive publication, the encyclopedia illustrates and emphasizes the fundamentals of the immune response. For immunity to play its part in good health, it must maintain homeostasis within itself and with all other physiologic systems. The challenges to maintaining immunologic good health are both internal and external. In the face of changes in the environment, including climate, infectious agents and industrial exposures, human survival places a need for constant recalibration of the immune system. Internally, the effects of aging,

hormonal changes, the microbiome and life cycle events (eg. puberty, pregnancy) also require readjustment of immunologic homeostasis. Interventions are designed to restore immunologic balance, to repair innate or induced deficiencies and to strengthen immune responses.

As Editors-in-Chief, we trust that the users will find this "encirclement" of a body of knowledge will prove helpful for decision making in promoting immunologic health and reducing immunologic disorders.

June 2014

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Preface

The immune system is the sensory organ that perceives and responds to dangerous alterations in tissue. The major alerts to the immune system are pathogen-associated molecular patterns (PAMPs) expressed by microbes and damage-associated molecular patterns (DAMPs) expressed by stressed or injured tissue. These triggers bind to membranes and cytosolic pattern recognition receptors (PRPs) and galvanize the immune system into action. An immune response begins with activation of an innate immune response and progresses to an adaptive immune response, which is the locus of immune memory. Interestingly, the response may also be communicated to the brain, which can contribute to the regulation of peripheral inflammation and immune responses.

During lymphocyte ontogeny and in the course of any immune response, there is a risk of the generation and/or activation of autoreactive lymphocytes that may cause an autoimmune disease. Development of autoimmune disease is regulated by the complement of genetic risk and protective alleles as well as by exposure to known and unknown environmental insults.

The encyclopedia will introduce the reader to processes of immune activation and quiescence that is required for self-tolerance. Both infectious and non-infectious mechanisms of immune activation are addressed. Moreover, tissue-specific immune function and immune pathologies are addressed in detail.

All authors are experts in their field, and all entries include a bibliography that provides further reading material. This encyclopedia is the first line in learning about specific immune mechanisms in health and disease. It has been designed to be useful to the new learner and to the expert alike.

June 2014

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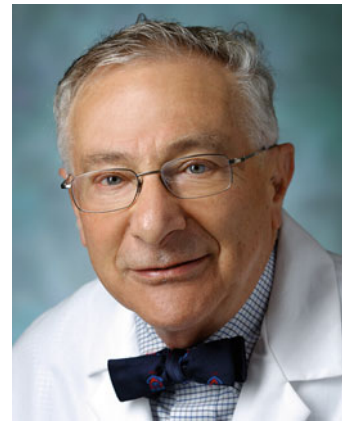
Editors-in-Chief

Photo credit:
Australian Academy of Science

Ian R. Mackay's research career, mostly directed to autoimmunity, began in 1956 in the Clinical Research Unit (CRU) of the Walter & Eliza Hall Institute and Royal Melbourne Hospital (RMH), Melbourne, Australia. It comprehended associations between disorders of immunological function and clinical expressions in diseases of obscure causation. Research laboratories in the Hall Institute and supervision of a 27-bed general medical ward in the adjacent RMH encouraged one to think of autoimmunity holistically rather than via any single disease. A particular interest in autoimmunity and liver and a collaboration with D Carleton Gajdusek pointed to autoimmune responses in causation of two major entities, chronic active hepatitis and primary biliary cirrhosis (PBC). The detection of autoimmune reactivity of a monoclonal plasma paraprotein was a key element in Burnet's formulation of the Clonal Selection Theory of Acquired Immunity. Mackay's later return to PBC in the molecular era (1980s) in research with M Eric Gershwin resulted in cloning and identification of the gene for the disease-associated "mitochondrial" autoantigen of PBC, the E2 subunit of pyruvate dehydrogenase complex (PDC-E2). In autoimmune hepatitis, levels in serum of transaminase enzymes were found to reflect ongoing hepatocellular damage, so providing a monitor of efficacy of immunosuppressive drugs prednisolone and azathioprine and, in the 1960s, the first long-term treatment trial established their benefit. This drug combination remains today as the standard therapy for autoimmune hepatitis.

In the early 1960s, Mackay became sufficiently convinced of the reality of autoimmunity to compile with F MacFarlane Burnet the first authoritative text (1963). Thereafter, he made research contributions on numerous autoimmune diseases, thyroiditis, multiple sclerosis, myasthenia gravis, pemphigus, and gastritis. With "Reg" Strickland, gastritis was separated into Type A (autoimmune) and Type B (later, bacterial) gastritis, foreshadowing bacterial infection in peptic ulcer disease. Mackay became a major protagonist for the early development of the specialty of Clinical Immunology and with Senga Whittingham laid out specifications for the practice of this specialty. In the 1980s, the RMH drew on the CRU to establish an AIDS service, and observations made on human papillomavirus (HPV) infection in rectal swabs of homosexual men led to Ian Frazer's development in Brisbane of an HPV vaccine for prevention of virus-induced cervical cancer.

In 1987, Mackay relocated to the Department of Biochemistry, Monash University, where with Merrill Rowley an autoimmunity laboratory was established for further investigation of PBC, Type 1 (autoimmune) diabetes, and rheumatoid arthritis. The laboratory sought to identify in various autoimmune diseases molecular epitopes (auto-epitopes) using contemporary techniques including antibody screening of phage-displayed random peptide libraries. A notable achievement arising from collaborations at Monash with James Whisstock, Gus Fenalti, and others was the crystallization of both isoforms of glutamic acid decarboxylase (GAD) 65 and 67, revealing the 3D structure and "molecular positioning" of the reactive antibody epitopes of the autoantigenic 65kD isoform and differences from the non-autoantigenic 67 kD isoform. This work is ongoing.



Noel R. Rose received his basic training in microbiology at Yale University followed by PhD and MD degrees at the University of Pennsylvania and State University of New York at Buffalo. He was appointed to the faculty at Buffalo in 1951, where he began his research career. His early studies under the tutelage of Professor Ernest Witebsky searched properties of the organ-specific antigens that characterize the unique functions of normal and malignant cells. In the course of these investigations, he discovered that he could produce an autoimmune disease in the thyroid gland by immunization with the major thyroid protein thyroglobulin. Until that time, it was generally accepted that in only a few "privileged sites" in the body were such pathogenic autoimmune responses possible. These studies opened the modern era of research on the autoimmune diseases and set the direction of Rose's career since that time. In the 1960s, he investigated the requisite conditions for inducing autoimmune disease and the delineation of the basic immunologic and pathological processes. He included studies on other organs, such as the pancreas, as well as allergic diseases. In 1971, he and his colleagues discovered the first major gene that is responsible for susceptibility to autoimmune diseases and proved that it was a member of the major histocompatibility complex. At that time, he moved his laboratory to Wayne State University in Detroit, where he and his colleagues carried out detailed studies on the genes responsible for autoimmune disease of the thyroid gland. He also performed early experiments of the regulatory role of the thymus-derived lymphocytes and other studies related to unique enzymes of specialized cells, especially prostatic cancer. In 1981, Rose moved to Johns Hopkins University, where he created a department devoted to studies of immunity and infection. He directed much of his research to infectious agents and chemicals that induce autoimmune disease. A major effort was devoted to developing an experimental model of autoimmune heart disease produced in genetically prepared mice by infection with a virus that led work to the first identification of a well-defined antigen responsible for cardiac inflammation. Investigations on this model revealed a stepwise process that leads from infection to initial harmless autoimmunity to later life-threatening autoimmune disease.

In addition to his research, Rose has been deeply involved in the clinical practice of immunology. He directs a diagnostic immunology laboratory; he

serves as expert consultant to the World Health Organization and as director of the WHO Collaborating Center for Autoimmune Disorders. He chaired the first committee on clinical immunology of the American Association of Immunologists and was co-founder of the Clinical Immunology Society. He was editor-in-chief of the first six volumes of the Manual of Clinical Immunology co-sponsored by the American Association of Immunologists and the American Society for Microbiology.

Throughout his career, Rose has had the opportunity of working with a number of leading investigators including Pierre Grabar at the Pasteuer Institute, Paris; Henry Isliker at the Swiss Institute for Cancer Research; Sir James Gowans at Oxford University; and Sir Gustav Nossal and Ian Mackay at the Walter and Eliza Hall Institute in Australia. While at the Hall Institute, Rose was invited to prepare a book describing the broad area of autoimmune disorders. He joined with Mackay in producing the first volume of the book, *The Autoimmune Diseases*, which is now in its fifth edition.

At Johns Hopkins, he continues to teach in medicine and public health and directs an active research laboratory. He also heads the Center for Autoimmune Disease Research, which facilitates communication and collaboration among specialists in the different facets of autoimmune disease research.

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Betty Diamond received an MD from Harvard Medical School in 1973. She performed a residency in Internal Medicine at Columbia Presbyterian Medical Center and then a postdoctoral fellowship in Immunology with Dr. Matthew Scharff at the Albert Einstein College of Medicine. She is currently Head of the Autoimmune Disease Center at the Feinstein Institute for Medical Research and on the faculty of the Albert Einstein College of Medicine.

Dr. Diamond's research has focused on the induction and pathogenicity of anti-DNA antibodies in Systemic Lupus Erythematosus. She received the Outstanding Investigator Award of the ACR in 2001, the Lee Howley Award from the Arthritis Foundation in 2002, and the Recognition Award from the National Association of MD-PhD Programs in 2004 and was elected to the Institute of Medicine in 2006. Dr. Diamond has served on the Scientific Council of NIAMS and the Board of Directors of the American College of Rheumatology. She is past president of the American Association of Immunologists.

Dr. Diamond's laboratory has demonstrated that a subset of anti-DNA antibodies cross-reacts with the NMDA receptor. These antibodies can mediate neuronal apoptosis in the hippocampus leading to a memory deficit or in the amygdala leading to a behavioral alteration. These antibodies are present in serum and cerebrospinal fluid and correlate with symptoms of neuropsychiatric lupus. These studies show that lupus antibodies can cause aspects of neuropsychiatric lupus in a noninflammatory fashion and create a paradigm for antibody-mediated changes in brain function in many conditions. With colleagues at the Feinstein Institute, she has generated a potential therapeutic to prevent neurotoxicity from these antibodies.

Anne Davidson, MBBS, The Feinstein Institute for Medical Research, NY, USA

Anne Davidson, received her MBBS degree from the University of Melbourne, Australia, and is a board-certified rheumatologist. She is currently an Investigator at the Feinstein Institute for Medical Research, New York, and Professor of Molecular Medicine at Hofstra North Shore-LIJ School of Medicine, New York, USA.

Dr. Davidson's research is focused on pathogenesis and therapy of SLE. She has worked extensively with mouse models of SLE, using newly discovered pathways of immune activation to determine the mechanisms of action of novel therapies for SLE. The results of these studies are then used to design mechanistic studies in the context of human SLE clinical trials. A main focus of the laboratory is to understand how B-cell tolerance is dysregulated in SLE. A second area of interest is to understand the mechanisms of inflammation within the SLE kidney, using a combination of systems biology and functional studies. She is a past recipient of the Dubois Award for SLE Research and the ACR Basic Science Distinguished Investigator Award.

Dr. Davidson is a member of the NIH study section PBKD and cochairs the grant review committee of the animal models subsection for the Lupus Research Institute. She is currently the Chair of the Scientific Advisory Council for the Rheumatology Research Foundation of the ACR.

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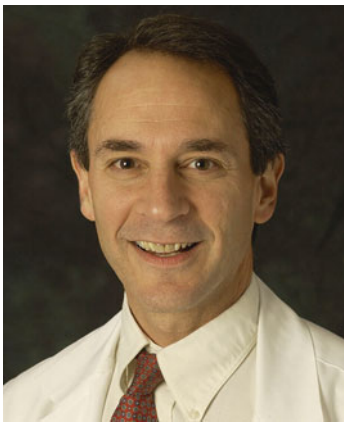

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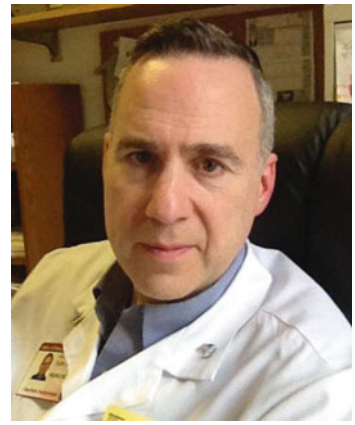

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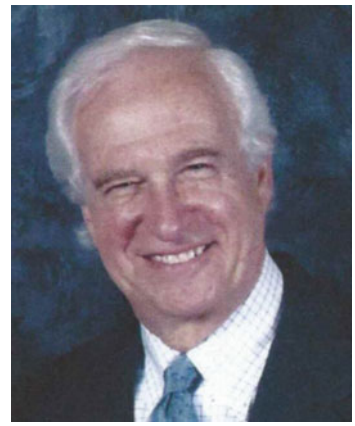

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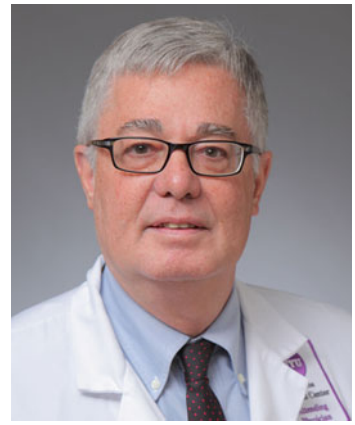

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A

Acute and Chronic Hepatitis B Virus Infection, Immune Response

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Synonyms

APC Antigen-presenting cell; CHB Chronic hepatitis B; HBV Hepatitis B virus

Definition

Hepatitis B virus (HBV) is a non-cytopathic DNA virus. The immune response to HBV is critically important to define, as it not only has potential to control the virus, but also to mediate liver inflammation and disease.

Immune Response in Acute Hepatitis B Virus Infection

The early immune events leading to clearance or persistence of HBV have been difficult to study. Unlike human immunodeficiency virus (HIV) and hepatitis C viruses (HCV), which are detectable in the periphery within days, HBV uniquely does not undergo a logarithmic phase of replication until 6–8 weeks after initial infection. Virus and viral proteins are undetectable during this incubation period. The clinical course of acute infection reflects this quiescent phase; patients develop symptoms only 10–12 weeks after initial infection or alternatively, remain asymptomatic throughout (Rehermann and Nascimbeni 2005). Therefore, a window of opportunity to study the kinetics of early innate and adaptive immune responses is often missed by the time patients are identified. Despite these setbacks, studies in human subjects identified incidentally (or after accidental inoculation) during the initial few weeks after virus encounter have provided insight into the early immune control of HBV.

Innate Immunity

The innate immune system provides a rapid, non-antigen-specific protection against pathogens, including a variety of viruses, bacteria, and fungi. It is the first line of defense not only in humans but in organisms as far removed as the *Drosophila* fly and *Caenorhabditis elegans* worm, which share evolutionary conserved receptors which recognise molecular patterns on the surface of pathogens as nonself (Akira et al. 2006).

During human infection, viral particles and their replicative intermediates are sensed by these pattern recognition receptors. These include Toll-like receptors, found on the surface and within endosomes of innate cells, and Rig-like helicases and Nod-like receptors within the cytosol. Production of antiviral type I interferons is the hallmark response. All nucleated cells have the capacity to produce these cytokines.

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Natural killer cells play an important role in the initial stages of antiviral immunity. They are the predominant lymphocytic subset within the liver and can kill virus-infected hepatocytes, cross-talk with dendritic cells and initiate priming of adaptive T cell responses and humoral immunity. In addition, a variety of non-parenchymal hepatic cells including Kupffer cells and liver sinusoidal endothelial cells contribute to viral sensing, making the liver an immunological organ (Bertoletti and Ferrari 2011).

Although this dogma of innate viral clearance is true for many viruses, gene-profiling studies have identified a stark lack of induction of type I IFN responsive genes in the liver of HBV-infected chimpanzees studied serially over the course of early acute infection (Wieland et al. 2005). Absence of type I interferon induction has also been confirmed in human studies of early acute infection (Dunn et al. 2009; Fisicaro et al. 2010). In a transgenic mouse model of HBV, type I interferons efficiently prevent the assembly of viral particles and destabilize existing viral capsids, suggesting that they do have the capacity to neutralize HBV particles when they are released. The absence of this critical innate effector function raises the possibility that HBV is a "stealth virus" which is able to evade innate immunity. Alternatively, there may be active mechanisms, which are suppressing innate immune responses during the initial phases of infection. The evidence for these two alternative hypotheses will be described in the following paragraph.

The idea that HBV avoids innate sensing mechanisms is supported by the fact that its transcriptional template (covalently closed circular DNA) remains within the nucleus of an infected cell and single-stranded RNA and DNA are shielded in viral capsids within the cytoplasm. These mechanisms may circumvent early detection by innate sensing receptors. However, data in human studies of early acute infection have provided compelling evidence that HBV does not completely evade innate immunity. Despite absence of type I interferon induction, several studies have shown an early peak in the frequency of NK and NT cells (T cells which express markers for classical NK cells) during the logarithmic phase of HBV infection and associated NK cell IFN-γ production and cytolytic activity (Fisicaro et al. 2009; Webster et al. 2004).

Alternatively, viral mechanisms may be suppressing early innate responses. HBV infected cells uniquely secretes milligram quantities of HBV proteins, HBsAg and HBeAg, which have no discernible role in viral replication. It is thought that these factors may play a role in modulation of immune responses through inhibition of Toll-like receptor signalling pathways or induction of immunosuppressive cytokines including IL-10. Study of acute infection has identified an early rise in serum IL-10 correlating with the logarithmic phase of viral replication and transient attenuation of NK and T cell responses (Dunn et al. 2009). Furthermore, the HBV X and polymerase proteins have been shown to interfere with type I interferon production (Bertoletti and Ferrari 2011).

Despite the apparent lack of a classical type I interferon response, the majority of patients who are acutely infected with HBV in adulthood resolve spontaneously. It is feasible that innate responses have a potential role in dampening initial viral responses during the incubation phase; however, late clinical presentation of patients has made timely sampling and study of this phase difficult.

Adaptive Immunity

HBV-specific adaptive immune responses are detectable 6–8 weeks after infection, correlating with the logarithmic phase of viral replication. They appear before onset of clinical symptoms and are critical mediators of viral clearance. A strong, polyfunctional, multi-specific T cell response is associated with self-limited infection. Patients who have established chronic infection characteristically have weaker HBV-specific responses, which are under constitutive negative regulation, although it has not been possible to determine whether these are the cause or result of chronicity.

CD4 T cells are required for priming and survival of functional CD8 T cell responses. They appear early during the logarithmic phase of

viremia; robust Th1 responses against multiple epitopes within the core protein have been associated with resolution of acute infection. Through production of IFN-γ and TNF, these cells contribute to non-cytolytic clearance of HBV but may also inadvertently potentiate liver inflammation. Evidence for their importance in early priming of CD8 T cell responses is suggested from chimpanzee studies, in which in vivo depletion of CD4 T cells prior to HBV inoculation resulted in dysfunctional CD8 T cell responses and ultimately, viral persistence (Asabe et al. 2009). CD4 T cells are also critical for induction of humoral immunity and generation of antibodies against the HBeAg.

The critical role for CD8 T cells in antiviral clearance is highlighted by depletion studies in chimpanzees. Depletion of CD8 T cells at the peak of viremia postponed viral clearance and development of hepatitis until reconstitution of T cells (Thimme et al. 2003). Their antiviral mechanisms are twofold. Since the majority of hepatocytes may be infected at peak viremia, non-cytolytic mechanisms, including production of the cytokines IFN-γ and TNF which can interfere with post-transcriptional steps of HBV replication, are important to prevent overwhelming organ damage (Guidotti et al. 1996). Additionally, cytolytic mechanisms include perforin-/granzyme-mediated lysis of infected hepatocytes. These initial mechanisms trigger the subsequent recruitment of a large non-antigen-specific lymphocytic infiltrate that contributes to liver inflammation and damage.

Of note, these findings apply to horizontally acquired adult infection. Over 95 % of individuals who acquire infection in this setting undergo viral resolution. In contrast, patients who are infected through vertical transmission have a much greater likelihood of developing chronic infection. The pattern of immune responses during acute infection following vertical exposure to HBV has not been well characterized.

Humoral Immunity

B cells play a prominent role during the symptomatic phase of infection, 10–12 weeks after initial inoculation. IgM antibodies to the HBV core antigen are a feature of early acute infection. IgG anti-HBc antibodies persist long term in both resolved and chronic infection whereas neutralizing antibodies directed to HBV surface antigen (anti-HBs) are associated with successful resolution of acute infection and contribute to protective immunity. The appearance of antibodies against the e antigen is also temporally associated with disease resolution, although their contribution to viral control is unclear. Furthermore, during chronic infection, production of anti-HBe antibodies marks a transition from a high to low replicative state. The protective potential of neutralizing antibodies is supported by the efficacy of the HBV vaccine; generation of antibodies against the surface antigen is associated with long-term protection against HBV infection. Additionally, the HBsAg used in the vaccine may also be cross-presented to prime T cell responses.

Immune Response in Chronic Hepatitis B Virus Infection

Despite the availability of a preventative vaccine, an estimated 350 million people are persistently infected with HBV according to the World Health Organisation (WHO). This leads to over one million annual deaths caused by HBV-related complications of liver cirrhosis and hepatocellular carcinoma (HCC). There is a 100-fold higher risk of HCC conferred by HBV infection (Beasley 1988); this is one of the top ten causes of mortality worldwide. Current antiviral therapies do not result in sustained off-treatment responses in the majority of individuals, therefore necessitating lifelong treatment and imposing a huge financial burden on the countries most affected. There is an urgent requirement for novel immunotherapeutic strategies which, in conjunction with current therapies, may tip the balance in favor of viral clearance.

Innate Immune Response

NK cells constitute around one third of intrahepatic lymphocytes. They have antiviral potential through their capacity to induce apoptosis of HBV-infected hepatocytes through the

tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathway (Dunn et al. 2007) or to non-cytolytically clear HBV via production of IFN-γ. Their antiviral contributions may be more significant in the setting of the exhausted CD8 T cell responses characteristic of CHB. However, NK cells are also partially dysfunctional in CHB, with impaired capacity to produce IFN-γ; these cells may be under negative regulation by immunosuppressive cytokines including IL-10 within the liver environment (Peppa et al. 2010).

Furthermore, NK cells have a dichotomous role in the liver. The effector functions required for antiviral activity also contribute to liver damage and immunopathology. Recruitment of NK cells to the liver as part of a larger non-antigen-specific infiltrate (triggered by HBV-specific mechanisms) may amplify inflammation through TRAIL-mediated hepatocyte damage and potentiate a pro-inflammatory environment.

Adaptive Immune Response

The capacity to detect HBV-specific T cell populations with a combination of intracellular cytokine staining and MHC class I restricted peptides/tetramers has facilitated functional profiling of these cells. These studies showed that HBV-specific CD4 and CD8 T cells are quantitatively depleted in the periphery and dysfunctional in their ability to proliferate and produce Th1/Tc1 type cytokines including IL-2 and IFN-γ in CHB. These weaker, narrowly focused responses fail to achieve adequate viral control.

Within HBV-specific CD8 T cells, an epitope hierarchy exists which varies with the different phases of chronic HBV infection. Core 18–27-specific CD8 T cells are dominant in patients with acute hepatitis (when compared to a limited panel of other HLA-A2-restricted epitopes inclusive of surface and polymerase proteins). Conversely, the same core 18–27-specific CD8 T cells are not detectable in the circulation in patients with CHB with viral loads $>10^7$ copies per ml (Webster et al. 2004); in these individuals, the few detectable responses are predominantly surface or polymerase-specific CD8 T cells. These dysfunctional specificities demonstrate altered tetramer binding and poor antiviral function and may represent persisting tolerized populations.

Although HBV utilizes the error-prone reverse transcriptase enzyme for replication, CTL escape mutations are thought to arise less frequently in HBV than in infections with other highly variable viruses such as HIV and HCV. This may be partially attributable to the weak selection pressure exerted on the virus by an exhausted HBV-specific CD8 T cell response in CHB but is likely also due to the constraints on sequence variation imposed by the overlapping reading frames of the HBV genome. T cell escape mutants have been observed in a few cases of CHB in whom the T cell response was uncharacteristically strong and narrowly focused (Bertoletti et al. 1994). Amino acid mutations have been described within HBcAg 18–27 epitope but not in surface and polymerase epitopes, suggesting a higher selection pressure by HBcAg-specific CD8 T cells.

The role of HBV-specific T cells within the liver has implications for liver immunopathogenesis in addition to antiviral immunity. The inadequate effector functions that fail to control viral load may instead potentiate liver inflammation. Release of IFN-γ has been shown to induce chemokine release by hepatocytes which recruit a large non-antigen-specific lymphocytic infiltrate into the liver (in a transgenic mouse model) (Bertoletti and Maini 2000). In this model of HBV infection, platelets have been found to play a novel role in the recruitment of CD8 T cells initiating HBV-related liver disease (Guidotti and Chisari 2006). Thus, functionally inadequate HBV-specific T cells which persist in CHB infection both fail to control viremia and, as a bystander effect, contribute to pathology (Bertoletti and Maini 2000). A large nonvirus-specific component to the T cell infiltrate has also been noted in patients with poorly controlled CHB (Maini et al. 2000). Many of the T cells constituting the intrahepatic cellular infiltrate in liver diseases such as HCV are now known to have a distinctive "liver-homing" phenotype, with expression of CD161, chemokine receptors such as CXCR6 or CXCR3, and an invariant Vα chain.

These mucosal-associated invariant T cells (MAIT) are prone to production of pro-inflammatory mediators such as IL-17, but their exact role in viral hepatitis remains to be elucidated (Klenerman and Thimme 2011).

Downregulation of the Antiviral T Cell Response in CHB

Virus-specific T cells are quantitatively depleted (inversely correlating with viral load) and profoundly dysfunctional in CHB. Their phenotype resembles that observed in the murine model of chronic LCMV infection, which has been termed T cell exhaustion. T cell exhaustion is characterized by a hierarchical loss of T cell effector functions. The capacity to produce IL-2 is lost early on, with an associated impairment of T cell proliferation. Cytotoxicity is lost next, followed by impaired production of effector cytokines TNF-α and IFN-γ, and finally deletion of the virus-specific T cells (Wherry 2011). In HBV infection, it has been shown that the loss of virus-specific T cells is associated with upregulation of the proapoptotic Bcl-2 interacting molecule (Bim) (Lopes et al. 2008). The few remaining T cells show a narrowly focused antiviral repertoire and in accordance with the exhaustion model, show no IL-2 production, weak or absent proliferative capacity, and impaired cytotoxicity and effector cytokine production. As IFN-γ has been shown to be a potent mediator of non-cytolytic clearance of HBV from hepatocytes, its lack of production is likely to contribute to viral persistence. HBV-specific T cells undergo phenotypic changes attenuating their functionality. The CD3-zeta signalling chain is downregulated as a hallmark of arginine deprivation, while co-inhibitory molecules such as programmed death 1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4) are upregulated. The expression of these co-inhibitory molecules thwarts any possible activation of the respective cells. Recent investigations have shown that blocking co-inhibitory pathways in vitro could help to recover T cell cytokine production by patient-derived T cells in many cases (Bertoletti and Ferrari 2011). These findings might help in the development of future immunomodulatory therapies.

The drivers contributing to the changes in phenotype and functional impairment of HBV-specific T cells are thought to be manifold. Firstly, the liver environment in which HBV-specific T cells are likely to encounter their cognate antigen has been described to be immunosuppressive, with low levels of arginine and high levels of the immunosuppressive cytokines IL-10 and TGF-β. Resident hepatic APC, such as liver sinusoidal endothelial cells (LSEC), have been shown to actively induce tolerance in antigen-specific T cells. Interaction with hepatocytes leads to the induction of a pro-apoptotic phenotype and T cell deletion. Many resident specialized intrahepatic cell types including LSEC, stellate cells, Kupffer cells, and hepatocytes themselves can deliver co-inhibitory signals to infiltrating T cells through their expression of ligands like PD-L1.

Secondly, high levels of persistent antigen can lead to tolerance induction in T cells, as shown in murine experiments. In addition to the very high levels of viremia persisting for years in many patients with CHB, HBV-derived antigens such as the HBeAg and subviral particles containing HBsAg are found in extremely high quantities in the circulation of these patients. The production of these noninfectious antigens is thought to constitute a viral immunosuppressive mechanism.

In addition to these mechanisms of T cell exhaustion, a number of other cell types may exert excessive downregulation of antiviral immunity in CHB. CD4 regulatory T cells (Treg) have the capacity to modulate immune responses by suppressing proliferation and effector functions in CD4 and CD8 T cells and interfering with the maturation of dendritic cells. Treg are important in the prevention of autoimmune responses; however, in chronic infections, they potentially hinder antiviral immune responses and consequently viral clearance. Despite numerous studies, there is still some controversy about the importance of CD4 T regulatory cells in HBV (Manigold and Racanelli 2007), which partly reflects the difficulty in reliably identifying these populations in humans. Fox-P3-expressing CD4 Treg are enriched in HBV infection, particularly in the liver, and have the capacity to suppress effector responses; they may therefore

constitute an additional mechanism to prevent excessive tissue damage by limiting the immune response in patients with high viral titers (Alatrakchi and Koziel 2009).

Conclusions

Despite the altered kinetics of HBV replication during the initial phases of infection, recent evidence suggests innate, in addition to adaptive, immune mediators may play a role in viral control. Further human studies and animal models of acute infection are required to define the immune events during the immediate few weeks after inoculation, details of which have remained elusive. Whether or not the endpoint of infection is viral persistence or control is likely decided during these initial few weeks.

Once chronic infection is established, it is perpetuated by a variety of mechanisms that normally serve to limit exuberant immune responses in the immunotolerant liver. A better understanding of these mechanisms has opened new avenues for developing therapeutic approaches to boosting immune control and limiting liver disease in CHB.

Cross-References

- Adaptive Immune Cells in the Liver
- Animal Models of Hepatitis B and C
- Bcl-2 Family Members and Lymphocyte Homeostasis
- CTLA-4
- Innate Immune Cells in the Liver
- Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance
- NK Cell Activation
- Primary T-Cell Activation in Liver
- Tregs in the Liver

References

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124:783–801.

Alatrakchi N, Koziel M. Regulatory T cells and viral liver disease. J Viral Hepat. 2009;16:223–9.

Asabe S, Wieland SF, Chattopadhyay PK, Roederer M, Engle RE, Purcell RH, Chisari FV. The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. J Virol. 2009;83:9652–62.

Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. Cancer. 1988;61:1942–56.

Bertoletti A, Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut. 2012;61(12):1754–64.

Bertoletti A, Maini MK. Protection or damage: a dual role for the virus-specific cytotoxic T lymphocyte response in hepatitis B and C infection? Curr Opin Immunol. 2000;12:403–8.

Bertoletti A, Sette A, Chisari FV, Penna A, Levrero M, De Carli M, Fiaccadori F, Ferrari C. Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells. Nature. 1994;369:407–10.

Dunn C, Brunetto M, Reynolds G, Christophides T, Kennedy PT, Lampertico P, Das A, Lopes AR, Borrow P, Williams K, et al. Cytokines induced during chronic hepatitis B virus infection promote a pathway for NK cell-mediated liver damage. J Exp Med. 2007;204:667–80.

Dunn C, Peppa D, Khanna P, Nebbia G, Jones M, Brendish N, Lascar RM, Brown D, Gilson RJ, Tedder RJ, et al. Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. Gastroenterology. 2009;137:1289–300.

Fisicaro P, Valdatta C, Boni C, Massari M, Mori C, Zerbini A, Orlandini A, Sacchelli L, Missale G, Ferrari C. Early kinetics of innate and adaptive immune responses during hepatitis B virus infection. Gut. 2009;58:974–82.

Fisicaro P, Valdatta C, Massari M, Loggi E, Biasini E, Sacchelli L, Cavallo MC, Silini EM, Andreone P, Missale G, Ferrari C. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. Gastroenterology. 2010;138:682–693.e4.

Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. Annu Rev Pathol. 2006;1:23–61.

Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity. 1996;4:25–36.

Klenerman P, Thimme R. T cell responses in hepatitis C: the good, the bad and the unconventional. Gut, 2012;61(8):1226–34.

Lopes AR, Kellam P, Das A, Dunn C, Kwan A, Turner J, Peppa D, Gilson RJ, Gehring A, Bertoletti A, Maini MK. Bim-mediated deletion of antigen-specific CD8 T cells in patients unable to control HBV infection. J Clin Invest. 2008;118:1835–45.

Maini MK, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, et al. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. J Exp Med. 2000;191:1269–80.

Manigold T, Racanelli V. T-cell regulation by CD4 regulatory T cells during hepatitis B and C virus infections: facts and controversies. Lancet Infect Dis. 2007;7:804–13.
Peppa D, Micco L, Javaid A, Kennedy PT, Schurich A, Dunn C, Pallant C, Ellis G, Khanna P, Dusheiko G, et al. Blockade of immunosuppressive cytokines restores NK cell antiviral function in chronic hepatitis B virus infection. PLoS Pathog. 2010;6:e1001227.
Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol. 2005;5:215–29.
Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, Chisari FV. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol. 2003;77:68–76.
Webster GJ, Reignat S, Brown D, Ogg GS, Jones L, Seneviratne SL, Williams R, Dusheiko G, Bertoletti A. Longitudinal analysis of CD8+ T cells specific for structural and nonstructural hepatitis B virus proteins in patients with chronic hepatitis B: implications for immunotherapy. J Virol. 2004;78:5707–19.
Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12:492–9.
Wieland SF, Eustaquio A, Whitten-Bauer C, Boyd B, Chisari FV. Interferon prevents formation of replication-competent hepatitis B virus RNA-containing nucleocapsids. Proc Natl Acad Sci USA. 2005;102:9913–7.

Adaptive Immune Cells in the Liver

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Synonyms

Hepatic adaptive immune cells

Definition

The adaptive immune cells of liver includes T cells and B cells, and they possess specificity, diversity, and immunological memory. These cells recognize specific antigenic epitopes. T lymphocytes provide cell-mediated immunity and consist of $CD8^+$ cytotoxic T lymphocytes (Tc) and $CD4^+$ T helper lymphocytes (Th). B lymphocytes are characterized by the production of antibodies raised against specific antigens and participate in humoral immunity. They orchestrate a major role of in both immunity and tolerance in the liver along with other immune cells.

The cardinal features of the adaptive immune system are specificity, diversity, and immunological memory. The adaptive (specific) immune response is orchestrated by T lymphocytes and B lymphocytes, which recognize specific antigenic epitopes. It has immunological memory in that a subsequent exposure to the same antigen leads to a more rapid, sustained, and potent immune response. T lymphocytes provide cell-mediated immunity and consist of $CD8^+$ cytotoxic T lymphocytes (Tc) and $CD4^+$T helper lymphocytes (Th). B lymphocytes are characterized by the production of antibodies raised against specific antigens and participate in humoral immunity. The normal liver contains a population of memory T cells that are involved in immune surveillance both locally and systemically. In response to infection or injury, innate and adaptive immune cells are rapidly recruited to the liver. Chronic hepatitis is characterized by hepatic infiltration with T and B effector cells that are crucial in determining whether inflammation resolves or persists with progression to cirrhosis. In viral hepatitis, liver-infiltrating T cells are crucial in controlling and clearing infection and defects in adaptive immunity lead to viral persistence and chronic hepatitis.

Introduction

The liver is the largest solid organ in the body and receives 75 % of its blood supply from the gut via the portal vein; the rest comes via the hepatic artery. The terminal branches of both drain into the hepatic sinusoids, a low-flow vascular bed where interactions with Kupffer cells, endothelial cells, and hepatocytes facilitate processing of nutrients and soluble antigens from the gut. The liver has an important role in the removal of

pathogens and particulate antigens entering from the systemic circulation via the hepatic artery and from the gut via the portal vein. The liver is thus constantly exposed to nutrients, antigens, and bacterial products and in order to prevent a constant state of immune activation. Mechanisms have evolved that suppress immune responses to harmless antigens while maintaining the capacity to respond to infections such as hepatitis viruses. In inflammatory and autoimmune liver diseases, lymphocytes are recruited, leading to hepatitis, which may become chronic if antigen persists, e.g., viral infection or autoimmune disease. Persistent chronic hepatitis can lead to progressive injury, an aberrant scarring, and cirrhosis. Thus, understanding how adaptive immune cells are activated, recruited, and positioned in the liver and how they respond to the hepatic microenvironment is critical to understanding the immune-pathogenesis of liver diseases.

The Adaptive Immune System

The adaptive immune system evolved to recognize and respond to a vast array of antigens and moreover to remember these antigens, leading to more rapid and vigorous responses on reexposure known as "immunological memory." The ability to recognize so many antigens is a consequence of accelerated somatic mutation and genetic recombination of antigen receptor gene segments which generates a vast number of potential antigen receptors (Siu et al. 1984). These are expressed on individual B and T lymphocytes and inherited by their progeny, allowing "memory" lymphocytes to deliver long-lived specific immunity. Teleologically, the adaptive immune system first appears in jawed fish and the innate immune system is the major system of host defense against pathogens in non-vertebrates (Medzhitov and Janeway 1997). However, the two systems are linked because activation of adaptive immune responses is dependent on signals generated in response to activation of pattern recognition receptors on antigen-presenting cells of the innate immune system by molecular motifs associated with pathogens or damaged cells. The cellular components of the innate immune system include antigen-presenting cells such as macrophages, dendritic cells, and some endothelial cells as well as eosinophils, basophils, and mast cells. In addition, epithelial cells can act as important sensors of tissue damage to activate innate lymphoid cells such as gamma delta T cells (Vantourout and Hayday 2013).

Lymphocytes are responsible for the specificity and memory in adaptive immune responses. They are produced in the bone marrow and mature within the thymus (T cells) or secondary lymphoid organs (B Cells) before emerging into the circulation as naive cells. These cells migrate between blood and secondary lymphoid tissue until they encounter their specific antigen. They express homing receptors that direct their migration between blood and secondary lymphoid tissues, allowing them to provide surveillance throughout the lymphatic tissues where they are most likely to encounter antigen in an environment that promotes optimal conditions for lymphocyte activation and the generation of immune responses (Miyasaka and Tanaka 2004).

CD8 αβ and CD4 T cells include a diverse repertoire of T cell receptors that recognize antigens in the context of MHC class I and II molecules, respectively. On activation by antigen presented by appropriately activated antigen-presenting cells (APCs), naive T cells differentiate into functional effector and memory cells with either T cytotoxic (Tc) or T helper (Th) cell functions (Kapsenberg 2003). Cytotoxic CD8 T cells recognize intracellular viral or tumor antigens presented in association with MHC class-I molecules whereas activated CD4 T cells secrete a range of cytokines depending on how and where they are activated that shape the nature of immune responses, e.g., Th1 responses dominated by Il-12, IFNγ and TNF secretion, Th2 dominated by IL-4 and IL-13, Th17 dominated by Il-17 secretion and Th22 cells that secrete IL-22. In addition, some CD4 T cells have regulatory functions and a specific subset of T follicular helper cells provides help to B cells (Vinuesa et al. 2005). An effective immune

response to most hepatotropic pathogens requires strong and durable CD4 and CD8 T cell responses that can be detected within the liver alongside regulatory T cells that are critical to control the effector immune response and allow resolution once the antigen is cleared (Protzer et al. 2012).

B cells produce antibodies that are secreted as soluble factors and recognize antigens as part of humoral immunity. B cells express a unique B cell receptor (BCR) that allows them to recognize specific antigen. Whereas T cells recognize their cognate antigen in a processed form presented by MHC molecules on APCs, B cells recognize antigen in the native form. If a B cell encounters its specific antigen in the context of additional signals provided by T cells (T cell help), it differentiates into an antibody-secreting plasma cell. Antibodies bind antigens and activate components of the innate immune system including phagocytes and complement. Some plasma cells become long-lived antigen-specific memory B cells primed to produce specific antibodies on reexposure to the pathogen (Table 1).

Adaptive Immune Cells in the Liver, Table 1 Characteristics of human T and B lymphocytes

	T lymphocytes	B lymphocytes
Maturation site	Thymus	Bone marrow
Surface markers	**CD3:** T cell receptor	Surface Ig, CD19
	CD4: Th1, Th2, Th17, Th22, Treg TFH	
	CD8: Tc (cytotoxic T cells)	
Intracellular cytokines	**Th1**: IFN-γ, TNF-α	Plasma cells secrete
	Th2: IL-4, IL-5, IL-13	IgG, IgM, IgA
	Th17: IL17, 22, GMCSF, IFN-γ	
	Th22: IL-22	
	Treg: IL-10	
Function	**Th1**: Kill intracellular microbes	Protect against extracellular microbes
	Th2: Parasitic infection	
	Th17: Fungal infection, autoimmunity	
	TFH: Provide helps for B cells	
	Treg: control other T cells	
Antigen receptor	TCR	BCR/Antibody
Memory cells	Yes	Yes
Antigen specificity	Yes	Yes
MHC recognition requirement	Yes	No

Activation of Adaptive Immunity in the Liver

The liver's well-known ability to promote immune tolerance has evolved to prevent excessive immune responses to nutrients or commensals entering via the portal vein. Some pathogens, for example, malaria and hepatitis C virus, exploit the liver's tolerogenic properties to evade effective immune responses and establish persistent infection. However, in most infections, the liver responds appropriately to pathogens by activating both the innate and adaptive immune responses to clear infections such as hepatitis A and E. The liver is characterized by populations of resident nonprofessional APCs in addition to conventional dendritic cells (DCs) both of which regulate local activation of T cells (Crispe 2012). The ability of liver sinusoidal endothelial cells (LSECs) and hepatocytes to interact with T cells is facilitated by the cellular composition and unique structure of hepatic sinusoids. Sinusoidal endothelial cells can take up, process, and present soluble antigens and apoptotic cells from the sinusoidal blood. Their characteristic fenestrations facilitate solute transport and allow hepatocytes to extend protrusions into the sinusoidal lumen (Warren et al. 2006) where as a consequence of low levels of flow, circulating lymphocytes can interact directly with endothelial cells, Kupffer cells, stellate cells, and hepatocytes during transit through the sinusoids (Benseler et al. 2011). LSEC can directly cross-present antigen to naive $CD8^+$

T cells, and this usually results in tolerance rather than full activation (Knolle et al. 1999; Limmer et al. 2000, 2005). The contribution of LSEC to intrahepatic $CD4^+$ T cell priming is less clear (Knolle et al. 1999; Limmer et al. 2000, 2005). Studies in mice suggest that LSECs have the ability to prime naïve lymphocytes with a regulatory phenotype (Knolle et al. 1999; Limmer et al. 2000), and a recent paper suggests that such activation can imprint responding cells with a gut homing phenotype, providing a potential link between the gut and liver (Neumann et al. 2012). Antigen presentation by hepatocytes leads to T cells entering hepatocytes and being degraded by a process called suicidal emperipolesis (Benseler et al. 2011). Antigen presentation by bone-marrow-derived APCs is regulated by the hepatic microenvironment to favor tolerance, and liver-derived DCs in mice and humans are inherently tolerogenic and express low levels of the co-stimulatory molecules required for full T cell activation (Goddard et al. 2004). Liver-derived DCs also preferentially secrete IL-10 and are capable of inducing peripheral Treg, which suppress immune responses (Thomson et al. 2009; Thomson 2010). Recent studies from the Bertolino group have shown that the liver microenvironment modulates the ability of bone-marrow-derived APC to activate viable effector T cells (Holz et al. 2012; Lopes et al. 2008; Sawa et al. 2009). Such an outcome is not seen in other tissues, suggesting that it is the liver microenvironment that determines this outcome. One factor may be the secretion of IL-7 by hepatocytes, which has been shown to regulate T cell survival (Sawa et al. 2009) or other cytokines such as IL-6, IL-10, or TGFβ secreted by stromal cells (Holt et al. 2009) or Kupffer cells.

The importance of local IL-10 secretion is illustrated by murine studies of the enteric pathogen *Trichinella spiralis* (Douglas et al. 2010). Wild type mice infected orally develop gut infection and local expansion of $CD4^+$ T cells but not hepatitis, whereas IL-10-deficient mice develop severe hepatitis mediated by $CD4^+$ T cells activated in the gut, illustrating the importance of local regulatory networks in the liver in protection against effector responses generated in the gut (Lunz et al. 2007). The constant exposure to bacterial products means that the liver is relatively insensitive to endotoxin, and Kupffer cells, and LSECs respond by preferentially secreting immunosuppressive cytokines, preventing the liver from being in a constant state of inflammation (Uhrig et al. 2005). Plasmacytoid DCs, which are recruited to the liver in response to inflammation, can also induce tolerance through anergy or apoptosis (Goubier et al. 2008) of antigen-specific $CD8^+$ T cells which may contribute to the elimination of antigen-specific cytotoxic T cells during the resolution phase of antiviral immunity.

In response to inflammation, myeloid DCs in the liver become activated and migrate via a parasinusoidal route from the parenchyma to portal tracts, which contain T cells and are therefore a potential site of T cell priming, before migrating to regional lymph nodes (Kudo et al. 1997). This route could explain the formation of portal lymphoid aggregates in many chronic inflammatory liver diseases including viral hepatitis.

Thus, under homeostatic conditions, antigen presentation within the liver favors immune tolerance and the outcome of intrahepatic immune responses is dependent on the local inflammatory microenvironment. When antigen is presented predominantly within the liver, $CD8^+$ T cell tolerance is likely to ensue whereas early antigen expression within secondary lymphoid tissue results in an effective cytotoxic T lymphocyte response.

Lymphocyte Recruitment to the Liver

Lymphocyte recruitment to the liver involves specific combinations of adhesion molecules and chemokines including the atypical endothelial adhesion molecule VAP-1 although no tissue-specific homing receptor has so far been reported for the liver. Circulating memory lymphocytes are widely distributed throughout the body via the blood to peripheral tissues such as the liver where they contribute to the process of immune

surveillance. The hepatic lymphocyte population also contains innate-like lymphoid cells, which include natural killer T cells, and γδ T cells, which play a critical role in first-line immune defense in response to injury or infection forming a bridge between innate and adaptive immunity. Around 15 % of $CD3^+$ lymphocytes from human liver express TCRγδ rather than TCRαβ.

Under normal conditions, lymphocyte recruitment to the liver is restricted to populations of memory T cells that recirculate as part of the process of immune surveillance. Such cells can be detected in portal tracts and scattered throughout the parenchyma and include both conventional lymphocytes, CD4 and CD8 T cells, T cells with αβ_ chain T cell receptors, that recognize antigens in the context of MHC class I and II molecules and a small number of memory B cells. In addition, NKT cells and populations of γδ T cells (both are known as unconventional T cells) and innate-like lymphoid cells including NK cells patrol the normal liver (Doherty and O'Farrelly 2000). Classical NKT cells express a very restricted T cell receptor repertoire Vα24 and Vβ11chains in humans and recognize antigens restricted to the MHC class I molecule CD1d that recognize antigen alpha-galactosyl ceramide. Nonclassical NKT cells encompass TCR_ αβ _ and TCRγ δ T cells, which do not use the T cell receptor Vα14 chain. Both classical and nonclassical NKT cells are abundant in the liver and constitute around 20–30 % of liver-infiltrating lymphocytes (Exley and Koziel 2004). Under non-inflamed conditions, liver-infiltrating lymphocyte comprise 60 % T cells, 30–35 % NK cells, and 5 % of B cells (Racanelli and Rehermann 2006). In response to infection or inflammation, recruitment increases rapidly, resulting in lymphocytic infiltrates in the portal tracts (portal hepatitis) or parenchyma (lobular hepatitis).

Lymphocytes are recruited from the circulation via sinusoidal endothelial cells, which express adhesion molecules and chemokines that support transendothelial migration. Subsequent interactions with stromal cells and extracellular matrix direct migration beyond the endothelium and into tissue. The recruitment of leukocytes to the liver occurs at three sites: (a) portal vein terminal branches, (b) central vein branches, and (c) hepatic sinusoids. Elegant studies in rodents suggest that most recruitment occurs through the sinusoids with subsequent migration onward into portal tracts and hepatic lymph (Xu et al. 2008).

In general, leukocyte recruitment via endothelium follows a four-stage process. Initially, free-flowing leukocytes are captured and tethered to the endothelium by selectins and their glycosylated ligands. However, in the low-flow system of the hepatic sinusoids, selectins have a much lesser role and other molecules including ICAM-1,VCAM-1, and VAP-1 may be involved at this stage (Lasky et al. 1992). Tethering induces rolling and the reduced velocity allows the cell to be activated by signals such as chemokines retained in the endothelial gycocalyx (Tanaka et al. 1993; Mackay 2001). This leads to the clustering and conformational activation of leucocytes integrins that support firm adhesion and arrest. Integrins are transmembrane heterodimers that mediate cell-cell and cell-matrix interactions and promote motility. They are required for transendothelial migration (Shimaoka et al. 2003). Integrins involved in leukocyte-endothelial interactions include αLβ2 (LFA-1) which binds to ICAM-1 and ICAM-2 on endothelium, α4β1 (VLA-4) which binds to VCAM-1 and α4β7 (LPAM-1) which binds to MadCAM-1, an adhesion molecule predominantly expressed in the gut (Butcher and Picker 1996). Integrins exist in an inactive conformation with low affinity for ligand until activated by signals such as chemokines (Campbell et al. 1998) (Table 2).

The adherent cell then undergoes intravascular crawling mediated by chemokines (Phillipson et al. 2006) and integrins followed by transendothelial migration through endothelium. Most transmigrating leukocytes migrate between endothelial cells at cell-cell junctions (paracellular migration) using junctional receptors including members of the JAM family (Nourshargh and Marelli-Berg 2005), but some leukocytes migrate through sinusoidal endothelium via transcellular pores within cells.

Adaptive Immune Cells in the Liver, Table 2 Integrins and their ligands, which are involved in recruitment of hepatic lymphocytes

Integrins on lymphocytes	Name	Ligands on sinusoid	Function
α4β1 (CD49d/CD29)	VLA-4,	VCAM-1, Fibronectin	Cell-matrix adhesion; lymphocyte adhesion
$\alpha_L\beta_2$ (CD11a/CD18)	LFA-1	ICAM-1 to ICAM-4	Leukocyte adhesion to sinusoid; transmigration; T cell-APC adhesion
α4β7 (CD49d/cd104)	LPAM-1	Fibronectin, VCAM-1, MadCAM-1	Lymphocyte homing to mucosal tissue

Both paracellular and transcellular migrations involve chemokines, integrins, and other atypical adhesion receptors including in the hepatic sinusoids both VAP-1 and for some leukocytes the scavenger receptor CLEVER-1 (Lalor et al. 2002; Shetty et al. 2011).

In most vascular beds, the leucocytes then has to cross the basement membrane although in the liver, this is absent and they enter the loose matricellular network of the space of Disse before entering the parenchyma. How cells traverse the space of Disse is poorly understood but probably involves interactions with stellate cells and extracellular matrix (Holt et al. 2009). Once in tissue, the leukocyte follows chemokine gradients to its target cell (Fig. 1).

Chemokines in the Liver

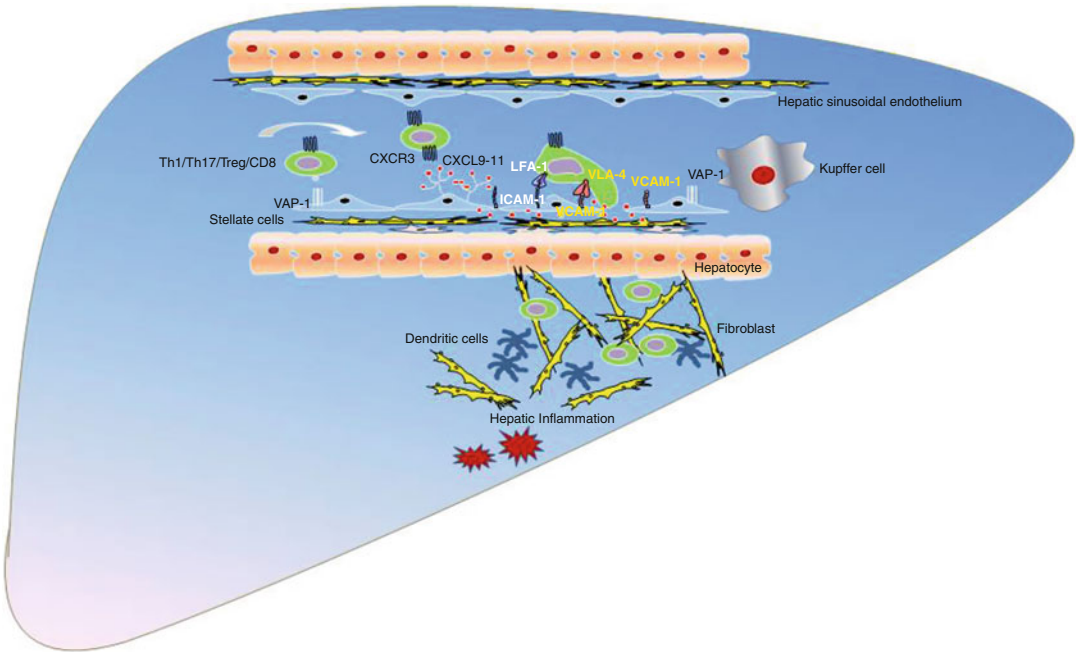
Chemokines are a family of structurally related chemotactic cytokines that regulate leukocyte migration, thereby shaping the outcome of immune responses. Chemokines provide cues for the recruitment of effector and regulatory subsets and are central to the pathogenesis of inflammatory diseases. They bind specific G protein–coupled receptors that activate integrin-mediated adhesion and enhance motility (Oo and Adams 2010). Chemokines are secreted early after infection or injury by endothelial and epithelial cells, stromal cells, and leukocytes and recruit an initial wave of innate immune cells that precedes antigen-specific effector and regulatory T cell recruitment.

Most effector T cells infiltrating the chronically inflamed human liver express high levels of CXCR3, CXCR6, CCR1, and CCR5 consistent with a tissue-infiltrating phenotype; the roles played by these different receptors in recruitment are beginning to be understood. In addition to recruiting different subsets of lymphocytes, there is evidence for compartmentalization of recruitment as well. CCR5 and its chemokine may have a particular role in recruitment to portal tracts, whereas CXCR3 appears to be essential for recruitment into the parenchyma (Table 3).

Chemokines in Lymphocyte Recruitment to the Liver

Expression of CXCR3 on T cells is closely linked to activation and tissue infiltration, and its ligands CXCL9, CXCL10, and CXCL11 are induced by proinflammatory cytokines. Studies have reported increased levels of CXCR3 on CD4 and CD8 effector cells within the liver with increased levels of hepatic CXCR3 ligands in many chronic inflammatory liver diseases. The sources of CXCR3 ligands in liver inflammation include hepatocytes, stellate cells, sinusoidal endothelial cells, and infiltrating leukocytes (Curbishley et al. 2005). The expression of CXCR3 ligands requires stimulation with IFN-γ and TNF-α, which are released by activated hepatic macrophages and the initial wave of infiltrating innate immune cells with an additional contribution from activated $CD4^+$ T cells themselves, thereby amplifying effector cell recruitment.

CXCR3 and two of its ligands CXCL9 and CXCL10 are important for transmigration of effectors T lymphocytes through hepatic sinusoids (Table 3). CXCR3 ligands are secreted by the endothelium but also originate from



Adaptive Immune Cells in the Liver, Fig. 1 The multistep model of leukocyte recruitment in the liver. Leukocyte extravasation into hepatic tissue involves multiple steps. First of all, lymphocytes are captured by carbohydrate-dependent tethering (which in the liver may be mediated by vascular adhesion protein-1 (VAP-1) rather than selectins which are absent from the hepatic sinusoids), allowing the flowing lymphocytes to contact the vessel wall where arrest can be triggered by chemokines CXCR3 on hepatic sinusoids immobilized on endothelial proteoglycans that activate lymphocyte integrins (LFA-1 and VLA-4) and binding to endothelial cell adhesion molecules ligands such as ICAM-1 and VCAM-1. During this stage, the leukocyte arrests on the vessel wall and undergoes intravascular crawling to sites of transendothelial migration. This process may involve migration between or through endothelial cells, and it is mediated by a number of molecular signals including chemokines, integrins, and atypical adhesion receptors such as VAP-1. Once in the tissue, the cell follows chemokine gradients presented on hepatic fibroblasts to sites of hepatic inflammation, using chemokine-mediated changes in the actin cytoskeleton, and adhesion molecules to propel migration

neighboring cells from where they can be transcytosed to the endothelial surface (Middleton et al. 2002). In addition, chemokines secreted "upstream" by other cell types including cholangiocytes in portal tracts can be captured from the slow-flowing sinusoidal blood by proteoglycans within the endothelial cell glycocalyx and presented at the endothelial surface. Thus, chemokines on the endothelium reflect local secretion by many cell types in the inflamed microenvironment (Edwards et al. 2005).

Chronic hepatitis includes nonspecific bystander lymphocytes as well as antigen-specific cells, and both express high levels of CXCR3. Although it was originally thought that CXCR3 expression was a characteristic of Th1 cells, it is now apparent that B cells, Tregs, and Th17 cells also use CXCR3 to enter inflamed tissues, including the liver, and CXCR3 may be viewed as important for tissue infiltration per se rather than being associated with particular types of inflammation.

Chemokines and Lymphocyte Subset Localization in the Liver

In human blood, CXCR6 is expressed on effector CD8 T cells with particularly high levels detected on CD4 and CD8 T cells within the inflamed human liver. The CXCR6 ligand CXCL16 is one of only two chemokines that exist in

Adaptive Immune Cells in the Liver, Table 3 Chemokines and their receptors involved in recruitment, positioning in the liver

Hepatic parenchyma cells	Chemokines	Responding chemokine receptors
Portal vessels	CCL3-5	CCR5
Liver sinusoids	CXCR9, 10, 11	CXCR3
	CXCL16	CXCR6
Biliary epithelium	CCL20	CCR6
	CCL28	CCR10
	CXCL16	CXCR6
Portal-associated lymphoid tissue	CCL19, 21	CCR7

Lymphocytes expressing CCR5 (Th1, CD8) can be recruited across the portal endothelium and this is mediated by the chemokines CCL3-5. Recruitment of lymphocyte subsets (CD8, Th1, Treg, and Th17) across liver sinusoidal endothelium is mediated by the chemokines CXCL9-11 as well as CXCL16 (NK and NKT). Biliary epithelial cells expressing CCL28 recruit CCR10-expressing regulatory T cells; CCL20 attract CCR6-expressing Th17 and CXCL16 attracts Th1, CD8, and NKT cells. In chronic hepatitis C and primary biliary cirrhosis, there is neovessel formation within the portal tracts, which has characteristics of lymphoid tissue, which is termed portal-associated lymphoid tissue (PALT) and has been shown to be associated with the chemokines CCL19 and CCL21 which are ligands for CCR7

a transmembrane form (Heydtmann et al. 2005). CXCL16 is upregulated on inflamed bile ducts and hepatocytes and is also expressed by sinusoidal endothelium where it mediates the patrolling of NK and NKT cells in the sinusoids (von Andrian and Mempel 2003; Geissmann et al. 2005). The engagement of CXCR6 on T cells by CXCL16 on epithelial cells promotes β1 integrin-dependent adhesion, which is important for the positioning, retention, and survival of effector cells in the inflamed liver (Germanov et al. 2008) (Table 3). A unique subset of HCV-specific $CXCR6^+$ liver-infiltrating CD8 T cells express the C-type lectin CD161 and secrete IL-17 and IFN-γ. These cells may represent an important liver-specific subset of effector cells (Billerbeck et al. 2010).

Other chemokines may also be involved in retaining T cells within the liver. These include CXCL12 (Terada et al. 2003) and the other transmembrane chemokine CX3CL1 (fractalkine), both of which are expressed on inflamed bile ducts (Isse et al. 2005). The fractalkine receptor CX3CR1 is expressed by Th1 cells and may help to retain these cells at sites of epithelial inflammation. The epithelial chemokine CCL28 is increased on cholangiocytes in a variety of liver diseases associated with T cells expressing the CCL28 receptor, CCR10 (Eksteen et al. 2006), a proportion of which are $FOXP3^+$ regulatory CD4 T cells. However, most liver-infiltrating Tregs express CXCR3 and CCR4, the latter allowing them to interact with chemokines secreted by hepatic dendritic cells.

Th17 and Tc17 cells express high levels of another chemokine receptor CCR6 and CCL20; the chemokine ligand for CCR6 is secreted by epithelial cells including cholangiocytes, providing a signal to localize Th17 cells at bile ducts at the epithelial surface and interface with the environment (Table 3).

Homeostatic Chemokines and Lymphocyte Egress From the Liver

Homeostatic chemokines can be upregulated at sites of inflammation where they play important roles in regulating leukocyte trafficking, particularly through the formation of tertiary neolymphoid structures (Grant et al. 2002) which are a feature of many chronic inflammatory liver diseases, particularly PBC, PSC, and HCV infection (Heydtmann et al. 2006). The CCR7 receptor is expressed on naive T cells and, a subset of central memory cells to promote their recirculation through secondary lymphoid tissues. CCR7 is also expressed on effector cells and is important for egressing out of inflamed tissue via the lymphatics (Debes et al. 2005). $CCR7^+$ effector T cells are present in inflamed human liver and because the CCR7 ligands CCL19 and CCL21 are expressed on hepatic sinusoids and lymphatic vessels (Grant et al. 2002), they may use CCR7 to migrate out of the liver via afferent lymphatics to draining lymph nodes where they are either removed during the resolution of infection or restimulated to maintain chronic hepatitis (Bromley et al. 2005).

T Lymphocytes Lineages in the Liver

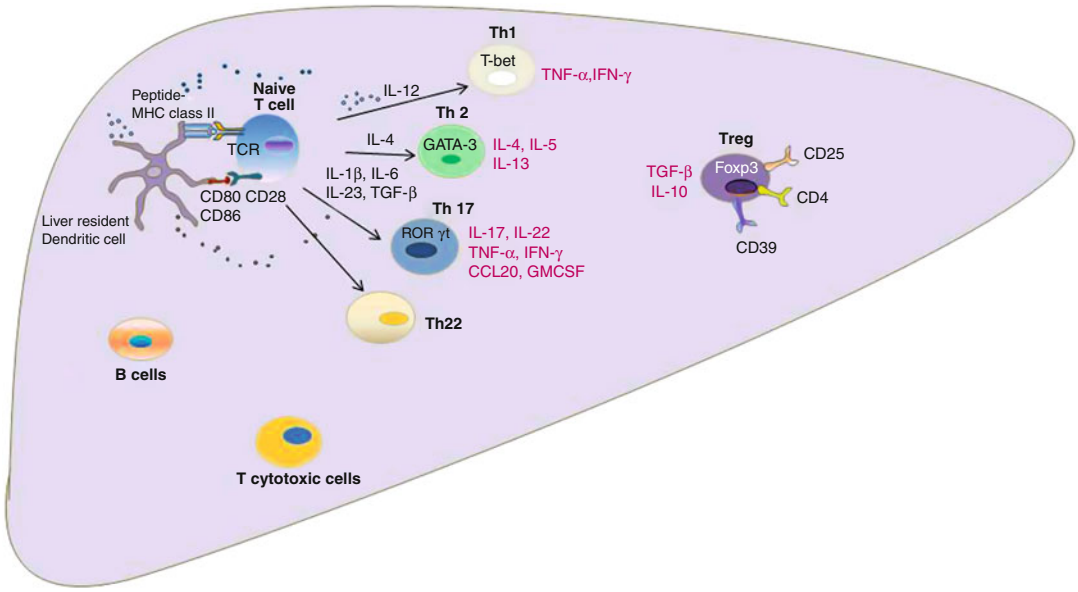
The normal human liver contains both CD4 and CD8 T cells with twice as many CD8 compared with CD4 T cells. Most conventional T cells in the non-inflamed liver are memory or effector phenotype and may be present as part of a circulating population of tissue-homing cells that provide immune surveillance against specific pathogens. The liver can act as a reservoir for antigen-specific T cells, and normal human liver contains memory cells with specificity for persistent viruses including CMV and EBV. In addition to conventional alpha beta T cells, the liver contains populations of *innate lymphoid cells* that include NK and NKT cells which play a role in immune surveillance against infection, epithelial injury, or malignant transformation (Mjosberg et al. 2012; Bernink et al. 2013). NKT cells are a subset of lymphocytes that express both the T cell receptor (TCR), specifically having an invariant VαJα-chain, and NK cell associated markers including NK1.1. NKT cells recognize glycolipid antigens presented by the non-polymorphic MHC class I-like protein CD1 in contrast to conventional $CD8^+$ and $CD4^+$ T cells that recognize peptide antigens presented by MHC. NKT cells are enriched in the human liver where they comprise nearly up to 20–30 % of hepatic lymphocytes whereas they make up less than 1 % of cells in other tissues. NKT cells are activated by glycolipid antigens presented by APCs or by cytokines such as IL-12, which is produced by dendritic cells and Kupffer cells. Subsequently, NKT cells secrete a large number of cytokines (IFN γ, TNFα, IL-4, IL-10, IL-13) and chemokines, allowing them to interact with the adaptive immune system and help determine the nature of the immune response.

Mucosal-associated invariant T (MAIT) cells are another subset of nonconventional αβ T cells whose T cell receptor (TCR) consists of an invariant Vα chain (iVα7.2-Jα33) combined with limited conserved Vβ chains that are restricted by a conserved MHC-related molecule MR1 (Gapin 2009). MAIT cells are present in human blood but use CCR6 and CXCR6 to preferentially locate to the lamina propria and liver (Gapin 2009). MAIT cells display active antimicrobial functions in vivo (Gold et al. 2010) and react with MR1-expressing cells infected or co-cultured with selective bacteria and yeasts, but not viruses (Gapin 2009). The activation of MAIT cells triggers secretion of IFN-γ, TNF-α, and IL-17 and again, they may have an important role in shaping immune responses in the liver.

In chronic hepatitis, both effector lymphocyte subsets and regulatory lymphocytes are present at the site of inflammation. The naive CD4 T cell is a multipotential precursor with defined antigen specificity, which can differentiate into distinct effector lineages, contingent upon signals from cells of the innate immune system. Th1 cells are crucial for bacterial and viral infection, Th2 for parasitic infection, and Th17 for fungal infection. Anti-inflammatory regulatory T cells (Treg) police these effector T cells and promote resolution of immune responses once infection has been cleared and help to maintain self-tolerance in the liver (Oo and Adams 2012). The balance of the cells recruited and their local differentiation and activation will determine the nature of hepatitis and liver disease (Fig. 2).

T cytotoxic Cells

Cytotoxic CD8 T cells are critical for antiviral and antitumor immunity. They kill target cells via activation of TNF receptors such as Fas and TRAIL and secretion of perforin and granzymes and also secrete proinflammatory cytokines including IFN-γ, TNF-α. They are present in normal human liver but increase in frequency in response to infection and are present in immune-mediated liver diseases where they may be associated with target structures such as bile ducts in PBC and hepatocytes in autoimmune hepatitis. Persistence of hepatitis B virus is associated with a failed CD8 T cells response which is a consequence of a combination of intrinsic T cell defects, particularly BIM-mediated apoptosis as a consequence of



Adaptive Immune Cells in the Liver, Fig. 2 T cell lineages in the liver. Once antigens are presented via MHC class II molecules and co-stimulatory molecule CD80/CD86, naive T cells differentiate into distinct CD4 helper T cell subsets, depending on the cytokine milieu, which drives a program of differentiation that includes the induction of key transcription factors, cytokine productions. Cytotoxic CD8 T cells and Th1 cells expressed inflammatory type chemokine receptors, CCR5& CXCR3; Th2 cells express CCR4 and CCR8; recently discovered Th17 cells express CCR4, CCR6, and CXCR3. Th22 subset was demonstrated in the liver, but chemokine receptors are still under investigation. Thymic derived regulatory T cells maintain the immune tolerance and prevent the excessive damage during infection and inflammation by controlling these T effectors cells. B cells are present in the liver and involved in humoral immunity by producing antibodies

activation by hepatic APCs, and a hepatic environment that favors apoptosis rather than survival of effector cells (Lopes et al. 2008). Liver-infiltrating CD8 cells express high levels of CXCR3 and CCR5, promoting their recruitment via endothelium, and also high levels of CXCR6 which allows them to be localized at and bind to epithelial targets that express the CXCR6 ligand CXCL16 (Berke 1995). In chronic liver inflammation, an exhausted phenotype similar to that seen in chronic HBV is suggested by downregulation of IFN-γ production along with upregulation of PD-1 on CD8 cells (Latchman et al. 2001).

Th1 and the Liver

Th1 immune responses are involved in cell-mediated immunity and the clearance of intracellular pathogens including viruses. They are also implicated in autoimmune and inflammatory liver diseases although in human liver disease, Th1, Th17, and even Th2 type cells can often be found together in the liver. Th1 cells differentiate when T cells are activated in the presence of dominant signaling through interleukin (IL)-12 and activation of signal transduction and activator of transcription (STAT)-4. They are characterized by expression of the transcription factor T-bet which promotes secretion of their signature cytokine IFN-γ. A critical factor in regulating the involvement of specific subsets at sites of inflammation is the differential expression of chemokine receptors between these different subsets of T cells. Liver-infiltrating CD8 T cells and Th1 both express high levels of CXCR3, CCR5, and CXCR6, promoting their recruitment to the inflamed liver (Curbishley et al. 2005; Heydtmann et al. 2006).

Th2 and the Liver

The differentiation of Th2 cells is driven by IL-4, STAT-6 and characterized by expression of the transcription factor Gata-3. They secrete Il-4, IL-5, IL-10, Il-13, and IL-21 and are involved in humoral immunity and the clearance of extracellular organisms and parasites including schistosomiasis (Cheever et al. 1995); (Wynn et al. 1998). IL-13 is a profibrotic cytokine implicated in Th2-dominated inflammatory response and subsequent liver fibrosis (Chiaramonte et al. 1999; Chiaramonte et al. 2001). IL-21 activates alternative macrophages and Kupffer cells and augments Th2 function and may have a role in hepatic fibrosis (Pesce et al. 2006).

Th17 and the Liver

Interleukin (IL)-17-producing Th17 cells have emerged as an independent T cell subset, whose differentiation requires IL-1, IL-6, and TGF-β, all of which are abundant in inflamed liver, activation of STAT3, and expression of the nuclear receptor, ROR-γt (Mosmann et al. 1986; Sakaguchi et al. 1995; Bettelli et al. 2006; Veldhoen et al. 2006; Annunziato et al. 2007). They are characterized by the secretion of IL-17 and IL-22 and can also secrete IFN-γ, GMCSF, and CCL20. They have been implicated in autoimmune diseases such as EAE and collagen-induced arthritis in mice (Mosmann et al. 1986; Sakaguchi et al. 1995; Bettelli et al. 2006; Veldhoen et al. 2006; Annunziato et al. 2007) and in the liver in steatohepatitis and autoimmune and viral liver disease (Lan et al. 2009; Lemmers et al. 2009; Tang et al. 2011; Zhao et al. 2011). Th17 cells link innate and adaptive immunity. The functional role of Th17 cells in the liver may be regenerative rather than pathogenic because their product IL-22 can promote epithelial repair and regeneration (Zenewicz et al. 2007). Th17 cells are present around peritumoral stroma of hepatocellular carcinoma (Kuang et al. 2010). Interleukin-17 signaling in murine models also promotes liver fibrosis (Gao and Waisman 2012; Meng et al. 2012).

Th22 and the Liver

Th22 cells mainly secrete IL-22, which is hepatoprotective in murine models (Xing et al. 2011). Il-22-secreting T cells can be detected in the inflamed human liver (Billerbeck et al. 2010; Kang et al. 2012), and some also express IL-17. The role of these cells is unclear, but they may be involved in repair and liver regeneration.

The recently reported Th9 cells that secrete IL-9 and develop from Th2 cells stimulated with IL-4 and TGF-β have not been reported in the liver.

Regulatory T Cells and the Liver

The efficient functioning of the immune system requires that effector responses against pathogens are tightly regulated to maintain homeostasis. Thymic derived naturally occurring $CD4^{+}CD25^{high}CD127^{low}$ regulatory T cells (T_{reg}) (Sakaguchi et al. 2008) are present and maintain tolerance in the liver. Hepatic regulatory T cells include natural Treg and induced Treg that develop in the TGF-β-rich intrahepatic cytokine milieu. Regulatory T cells are present in acute and chronic viral, autoimmune liver diseases and also in hepatocellular carcinoma (Longhi et al. 2005; Fu et al. 2007; Stross et al. 2012). Treg suppress immune response against self-antigens and also damp down effector responses to allow resolution and the restoration of immune homeostasis, thereby preventing autoimmunity and uncontrolled inflammation and tissue injury. Their function depends on activation of STAT-5 via IL-2 signaling. Liver-infiltrating Treg express patterns of homing receptors that overlap with T effector cells, Th1 cells (CXCR3, CCR5 and CXCR6), Th2 cells (CCR4 and CCR8), and Th-17 cells (CCR4, CCR6, CXCR3), allowing Treg cells to co-localize together with diverse effector T cells to suppress a wide range of inflammatory conditions (Curbishley et al. 2005). Treg in the inflamed human liver use CXCR3 to migrate through sinusoidal endothelium under with other receptors localizing them at epithelial surfaces (CCR10) or close to DCs (CCR4) (Eksteen et al. 2006).

The function of hepatic Treg may be impaired by the local inflamed microenvironment, as demonstrated by impaired phosphorylation of STAT5 (Oo et al. 2010). Recent studies implicated Treg surface markers CTLA-4, CD39 and Galactin-9 may play a role in liver diseases (Deaglio et al. 2007; Qureshi et al. 2011; Liberal et al. 2012).

B Lymphocytes in the Liver

B cells are present in chronically inflamed liver and use a combination of VCAM-1, ICAM-1, and two atypical adhesion receptors, VAP-1 and CLEVER-1, to enter the liver (Shetty et al. 2012). Hepatic lymphoid follicles with clear B and T cell areas develop in many chronic inflammatory diseases, usually close to portal tracts, leading to them being termed portal-associated lymphoid tissues (PALT). Activated B cells are surrounded by a follicular dendritic cells network and the distribution of IgM, IgD, IgG B cells and of bcl-2 and bcl-6 resembles that seen in lymph nodes (Racanelli et al. 2001). B cells also play their part in hepatic fibrosis as attenuated liver fibrosis was noted in the absence of B cells (Novobrantseva et al. 2005).

Utilizing the Adaptive Immune Cells in Liver Disease

Increasing understanding of the role played by specific chemokines and adhesion molecules in regulating adaptive immune cell recruitment to the liver opens up the chance of manipulating these pathways to prevent the recruitment of damaging effector cells or promote recruitment of anti-inflammatory regulatory cells. Potential therapeutic targets include CXCR3 and CXCR6 in inflammatory liver disease. Augmenting the immunoregulatory arm by supplementing autologous regulatory T cells to suppress unwanted inflammation is attractive in autoimmune disease and has been achieved in part by treating patients with IL-2 to activate Tregs. Another approach is to expand Treg ex vivo and re-infuse them into patients. In the context of liver cancer, Tregs suppress beneficial antitumor immunity and here Treg depletion is the aim. This can be achieved in part by judicious use of drugs such as cyclophosphamide but specific Treg-depleting therapy is missing. However, such approaches are complex, because it is difficult to specifically target either the regulatory or effector arm with current therapies. Further preclinical studies and better animal models are necessary to allow the development of safe and effective clinical therapies.

Cross-References

- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Cytotoxic T Lymphocytes
- ▶ Tregs in the Liver

References

Annunziato F, Cosmi L, et al. Phenotypic and functional features of human Th17 cells. J Exp Med. 2007;204(8):1849–61.

Benseler V, Warren A, et al. Hepatocyte entry leads to degradation of autoreactive CD8 T cells. Proc Natl Acad Sci USA. 2011;108(40):16735–40.

Berke G. The CTL's kiss of death. Cell. 1995;81(1):9–12.

Bernink JH, Peters CP, et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nat Immunol. 2013;14:221–9.

Bettelli E, Carrier Y, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441(7090):235–8.

Billerbeck E, Kang YH, et al. Analysis of CD161 expression on human CD8+ T cells defines a distinct functional subset with tissue-homing properties. Proc Natl Acad Sci USA. 2010;107(7):3006–11.

Bromley SK, Thomas SY, et al. Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. Nat Immunol. 2005;6(9):895–901.

Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science. 1996;272(5258):60–6.

Campbell JJ, Hedrick J, et al. Chemokines and the arrest of lymphocytes rolling under flow conditions. Science. 1998;279(5349):381–4.

Cheever AW, Finkelman FD, et al. Anti-interleukin-4 treatment diminishes secretion of Th2 cytokines and inhibits hepatic fibrosis in murine schistosomiasis japonica. Parasite Immunol. 1995;17(2):103–9.

Chiaramonte MG, Donaldson DD, et al. An IL-13 inhibitor blocks the development of hepatic fibrosis during

a T-helper type 2-dominated inflammatory response. J Clin Invest. 1999;104(6):777–85.
Chiaramonte MG, Cheever AW, et al. Studies of murine schistosomiasis reveal interleukin-13 blockade as a treatment for established and progressive liver fibrosis. Hepatology. 2001;34(2):273–82.
Crispe IN. Migration of lymphocytes into hepatic sinusoids. J Hepatol. 2012;57(1):218–20.
Curbishley SM, Eksteen B, et al. CXCR3 activation promotes lymphocyte transendothelial migration across human hepatic endothelium under fluid flow. Am J Pathol. 2005;167(3):887–99.
Deaglio S, Dwyer KM, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med. 2007;204(6):1257–65.
Debes GF, Arnold CN, et al. Chemokine receptor CCR7 required for T lymphocyte exit from peripheral tissues. Nat Immunol. 2005;6(9):889–94.
Doherty DG, O'Farrelly C. Innate and adaptive lymphoid cells in the human liver. Immunol Rev. 2000;174:5–20.
Douglas DB, Beiting DP, et al. Combinatorial effects of interleukin 10 and interleukin 4 determine the progression of hepatic inflammation following murine enteric parasitic infection. Hepatology. 2010;51(6):2162–71.
Edwards S, Lalor PF, et al. Lymphocyte traffic through sinusoidal endothelial cells is regulated by hepatocytes. Hepatology. 2005;41(3):451–9.
Eksteen B, Miles A, et al. Epithelial inflammation is associated with CCL28 production and the recruitment of regulatory T cells expressing CCR10. J Immunol. 2006;177(1):593–603.
Exley MA, Koziel MJ. To be or not to be NKT: natural killer T cells in the liver. Hepatology. 2004;40(5):1033–40.
Fu J, Xu D, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology. 2007;132(7):2328–39.
Gao B, Waisman A. Th17 cells regulate liver fibrosis by targeting multiple cell types: many birds with one stone. Gastroenterology. 2012;143(3):536–9.
Gapin L. Where do MAIT cells fit in the family of unconventional T cells? PLoS Biol. 2009;7(3):e70.
Geissmann F, Cameron TO, et al. Intravascular immune surveillance by CXCR6+ NKT cells patrolling liver sinusoids. PLoS Biol. 2005;3(4):e113.
Germanov E, Veinotte L, et al. Critical role for the chemokine receptor CXCR6 in homeostasis and activation of CD1d-restricted NKT cells. J Immunol. 2008;181(1):81–91.
Goddard S, Youster J, et al. Interleukin-10 secretion differentiates dendritic cells from human liver and skin. Am J Pathol. 2004;164(2):511–9.
Gold MC, Cerri S, et al. Human mucosal associated invariant T cells detect bacterially infected cells. PLoS Biol. 2010;8(6):e1000407.
Goubier A, Dubois B, et al. Plasmacytoid dendritic cells mediate oral tolerance. Immunity. 2008;29(3):464–75.
Grant AJ, Goddard S, et al. Hepatic expression of secondary lymphoid chemokine (CCL21) promotes the development of portal-associated lymphoid tissue in chronic inflammatory liver disease. Am J Pathol. 2002;160(4):1445–55.
Heydtmann M, Lalor PF, et al. CXC chemokine ligand 16 promotes integrin-mediated adhesion of liver-infiltrating lymphocytes to cholangiocytes and hepatocytes within the inflamed human liver. J Immunol. 2005;174(2):1055–62.
Heydtmann M, Hardie D, et al. Detailed analysis of intrahepatic CD8 T cells in the normal and hepatitis C-infected liver reveals differences in specific populations of memory cells with distinct homing phenotypes. J Immunol. 2006;177(1):729–38.
Holt AP, Haughton EL, et al. Liver myofibroblasts regulate infiltration and positioning of lymphocytes in human liver. Gastroenterology. 2009;136(2):705–14.
Holz LE, Benseler V, et al. Naive CD8 T cell activation by liver bone marrow-derived cells leads to a "neglected" IL-2low Bimhigh phenotype, poor CTL function and cell death. J Hepatol. 2012;57(4):830–6.
Isse K, Harada K, et al. Fractalkine and CX3CR1 are involved in the recruitment of intraepithelial lymphocytes of intrahepatic bile ducts. Hepatology. 2005;41(3):506–16.
Kang YH, Seigel B, et al. CD161(+)CD4(+) T cells are enriched in the liver during chronic hepatitis and associated with co-secretion of IL-22 and IFN-gamma. Front Immunol. 2012;3:346.
Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol. 2003;3(12):984–93.
Knolle PA, Schmitt E, et al. Induction of cytokine production in naive CD4(+) T cells by antigen- presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. Gastroenterology. 1999;116(6):1428–40.
Kuang DM, Peng C, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma promote expansion of memory T helper 17 cells. Hepatology. 2010;51(1):154–64.
Kudo S, Matsuno K, et al. A novel migration pathway for rat dendritic cells from the blood: hepatic sinusoids-lymph translocation. J Exp Med. 1997;185(4):777–84.
Lalor PF, Edwards S, et al. Vascular adhesion protein-1 mediates adhesion and transmigration of lymphocytes on human hepatic endothelial cells. J Immunol. 2002;169(2):983–92.
Lan RY, Salunga TL, et al. Hepatic IL-17 responses in human and murine primary biliary cirrhosis. J Autoimmun. 2009;32(1):43–51.
Lasky LA, Singer MS, et al. An endothelial ligand for L-selectin is a novel mucin-like molecule. Cell. 1992;69(6):927–38.

Latchman Y, Wood CR, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol. 2001;2(3):261–8.

Lemmers A, Moreno C, et al. The interleukin-17 pathway is involved in human alcoholic liver disease. Hepatology. 2009;49(2):646–57.

Liberal R, Grant CR, et al. The impaired immune regulation of autoimmune hepatitis is linked to a defective Galectin-9/Tim-3 pathway. Hepatology. 2012;56:677–86.

Limmer A, Ohl J, et al. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med. 2000;6(12):1348–54.

Limmer A, Ohl J, et al. Cross-presentation of oral antigens by liver sinusoidal endothelial cells leads to CD8 T cell tolerance. Eur J Immunol. 2005;35(10):2970–81.

Longhi MS, Ma Y, et al. Effect of CD4+ CD25+ regulatory T-cells on CD8 T-cell function in patients with autoimmune hepatitis. J Autoimmun. 2005; 25(1):63–71.

Lopes AR, Kellam P, et al. Bim-mediated deletion of antigen-specific CD8 T cells in patients unable to control HBV infection. J Clin Invest. 2008;118(5):1835–45.

Lunz III JG, Specht SM, et al. Gut-derived commensal bacterial products inhibit liver dendritic cell maturation by stimulating hepatic interleukin-6/signal transducer and activator of transcription 3 activity. Hepatology. 2007;46(6):1946–59.

Mackay CR. Chemokines: immunology's high impact factors. Nat Immunol. 2001;2(2):95–101.

Medzhitov R, Janeway Jr CA. Innate immunity: the virtues of a nonclonal system of recognition. Cell. 1997;91(3):295–8.

Meng F, Wang K, et al. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology. 2012;143(3):765–76 e761-763.

Middleton J, Patterson AM, et al. Leukocyte extravasation: chemokine transport and presentation by the endothelium. Blood. 2002;100(12):3853–60.

Miyasaka M, Tanaka T. Lymphocyte trafficking across high endothelial venules: dogmas and enigmas. Nat Rev Immunol. 2004;4(5):360–70.

Mjosberg J, Bernink J, et al. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. Immunity. 2012;37(4):649–59.

Mosmann TR, Cherwinski H, et al. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986;136(7):2348–57.

Neumann K, Kruse N, et al. Connecting liver and gut: murine liver sinusoidal endothelium induces gut tropism of CD4+ T cells via retinoic acid. Hepatology. 2012;55(6):1976–84.

Nourshargh S, Marelli-Berg FM. Transmigration through venular walls: a key regulator of leukocyte phenotype and function. Trends Immunol. 2005;26(3):157–65.

Novobrantseva TI, Majeau GR, et al. Attenuated liver fibrosis in the absence of B cells. J Clin Invest. 2005;115(11):3072–82.

Oo YH, Adams DH. The role of chemokines in the recruitment of lymphocytes to the liver. J Autoimmun. 2010;34(1):45–54.

Oo YH, Adams DH. Regulatory T cells and autoimmune hepatitis: defective cells or a hostile environment? J Hepatol. 2012;57(1):6–8.

Oo YH, Weston CJ, et al. Distinct roles for CCR4 and CXCR3 in the recruitment and positioning of regulatory T cells in the inflamed human liver. J Immunol. 2010;184(6):2886–98.

Pesce J, Kaviratne M, et al. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. J Clin Invest. 2006;116(7):2044–55.

Phillipson M, Heit B, et al. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. J Exp Med. 2006;203(12):2569–75.

Protzer U, Maini MK, et al. Living in the liver: hepatic infections. Nat Rev Immunol. 2012;12(3):201–13.

Qureshi OS, Zheng Y, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science. 2011;332(6029):600–3.

Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology. 2006;43(2 Suppl 1):S54–62.

Racanelli V, Sansonno D, et al. Molecular characterization of B cell clonal expansions in the liver of chronically hepatitis C virus-infected patients. J Immunol. 2001;167(1):21–9.

Sakaguchi S, Sakaguchi N, et al. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155(3):1151–64.

Sakaguchi S, Yamaguchi T, et al. Regulatory T cells and immune tolerance. Cell. 2008;133(5):775–87.

Sawa Y, Arima Y, et al. Hepatic interleukin-7 expression regulates T cell responses. Immunity. 2009;30(3):447–57.

Shetty S, Weston CJ, et al. Common lymphatic endothelial and vascular endothelial receptor-1 mediates the transmigration of regulatory T cells across human hepatic sinusoidal endothelium. J Immunol. 2011;186(7):4147–55.

Shetty S, Bruns T, et al. Recruitment mechanisms of primary and malignant B cells to the human liver. Hepatology. 2012;56:1521–31.

Shimaoka M, Xiao T, et al. Structures of the alpha L I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. Cell. 2003;112(1):99–111.

Siu G, Clark SP, et al. The human T cell antigen receptor is encoded by variable, diversity, and joining gene segments that rearrange to generate a complete V gene. Cell. 1984;37:393–401.

Stross L, Gunther J, et al. Foxp3+ regulatory T cells protect the liver from immune damage and compromise

virus control during acute, experimental hepatitis B virus infection. Hepatology. 2012;56:873–83.
Tanaka Y, Adams DH, et al. T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1 beta. Nature. 1993;361(6407):79–82.
Tang Y, Bian Z, et al. Interleukin-17 exacerbates hepatic steatosis and inflammation in non-alcoholic fatty liver disease. Clin Exp Immunol. 2011;166(2):281–90.
Terada R, Yamamoto K, et al. SDF-1 from biliary cells recruits CXCR4-positive cells: implications for inflammatory liver diseases. Lab Invest. 2003;83(5):665–72.
Thomson AW. Tolerogenic dendritic cells: all present and correct? Am J Transplant. 2010;10:214–9.
Thomson AW, Turnquist HR, et al. Tolerogenic dendritic cell-regulatory T-cell interaction and the promotion of transplant tolerance. Transplantation. 2009;87 Suppl 9:S86–90.
Uhrig A, Banafsche R, et al. Development and functional consequences of LPS tolerance in sinusoidal endothelial cells of the liver. J Leukoc Biol. 2005;77(5):626–33.
Vantourout P, Hayday A. Six-of-the-best: unique contributions of gammadelta T cells to immunology. Nat Rev Immunol. 2013;13(2):88–100.
Veldhoen M, Hocking RJ, et al. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity. 2006;24(2):179–89.
Vinuesa CG, Tangye SG, et al. Follicular B helper T cells in antibody responses and autoimmunity. Nat Rev Immunol. 2005;5(11):853–65.
von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. Nat Rev Immunol. 2003;3(11):867–78.
Warren A, Le Couteur DG, et al. T lymphocytes interact with hepatocytes through fenestrations in murine liver sinusoidal endothelial cells. Hepatology. 2006;44(5):1182–90.
Wynn TA, Cheever AW, et al. IL-10 regulates liver pathology in acute murine Schistosomiasis mansoni but is not required for immune down-modulation of chronic disease. J Immunol. 1998;160(9):4473–80.
Xing WW, Zou MJ, et al. Hepatoprotective effects of IL-22 on fulminant hepatic failure induced by D-galactosamine and lipopolysaccharide in mice. Cytokine. 2011;56(2):174–9.
Xu XD, Ueta H, et al. Trafficking of recirculating lymphocytes in the rat liver: rapid transmigration into the portal area and then to the hepatic lymph. Liver Int. 2008;28(3):319–30.
Zenewicz LA, Yancopoulos GD, et al. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. Immunity. 2007;27(4):647–59.
Zhao L, Tang Y, et al. Interleukin-17 contributes to the pathogenesis of autoimmune hepatitis through inducing hepatic interleukin-6 expression. PLoS One. 2011;6(4):e18909.

Alopecia Areata

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Synonyms

Non-scarring hair loss; Patchy alopecia; Patchy hair loss

Alopecia areata (AA) is a disease which causes hair loss commonly on the scalp and much less frequently over other hair-bearing areas of the body (e.g., eyebrows, beard). AA has a prevalence of 0.1–0.2 % with a lifetime risk of 1.7 % in the general population (Safavi 1992). It affects individuals of all ages, gender, and ethnicity.

It is a cutaneous disease which may have nail changes. However, other organ systems are not affected. Even in the most severe sequelae of entire scalp and body hair loss, this disease does not portend a serious medical problem; however, the psychological and social impact can be immense.

There is increased incidence of AA among patients with genetically related persons having this disease (10–20 %) (Freyschmidt-Paul et al. 2010).

This condition has been associated with a personal history of atopy and has been found to occur with other autoimmune diseases such as lupus erythematosus, vitiligo, type I diabetes mellitus (Wang et al. 1994), and autoimmune thyroid disorders (Kurtev and Iliev 2005).

Pathogenesis

The exact etiopathogenesis of AA is not fully known. There is overwhelming evidence

supporting the major role of the immune system in the pathogenesis of this disease. At present, AA is considered an autoimmune disease based on substantial research data though some crucial findings to establish true autoimmunity (e.g., identification of primary target self-hair-follicle-derived antigen/s; reactivity to self-follicular antigens leading to AA in humans) are still lacking (Freyschmidt-Paul et al. 2010).

In AA, inflammatory infiltrates are found mainly over the peribulbar region of the terminal anagen hair follicle (Whiting 2003; Dy 2011; Alkalifah et al. 2010).

The inflammatory attack does not occur over the region of the epithelial follicular stem cells (bulge area); thus, permanent damage is not seen in this disease and hair regrowth is possible.

Research findings indicate that cellular-mediated immune system plays a significant role in the pathogenesis of AA. The inflammatory infiltrate in AA consists of T cells mainly CD4 and CD8. CD4+ T lymphocytes are mainly found surrounding hair follicles, while CD8+ cells are located intrafollicularly (Freyschmidt-Paul et al. 2010; Carroll et al. 2002; McElwee et al. 2002). The activated CD8+ infiltrating inside hair follicles causes cytotoxic reaction, resulting in follicular damage. Molecules produced by cytotoxic T cells include tumor necrosis factor (TNF), granzymes, and Fas ligand (FasL) (Carroll et al. 2002; McElwee et al. 2002). Fas are highly expressed in dystrophic AA hair follicles (Bodemer et al. 2000; McElwee and Hoffman 2002). Interferon gamma (IFN gamma) produced by follicular antigen-presenting cells and mediated by CD4 T helper cells has been shown to be elevated in AA patients especially in alopecia areata totalis or universalis. This cytokine has been found to precipitate hair loss in AA (Freyschmidt-Paul et al. 2006; Arca et al. 2004).

The pathogenic role of T lymphocytes (mainly CD8 and CD4) is further supported by the induction of AA in animal models through the transfer of particular cells and grafting from AA mice to normal mice, leading to the development of patchy hair loss (McElwee et al. 2005; Freyschmidt-Paul et al. 2008). In murine models, transferred CD8+ induced localized disease, whereas CD4+/CD25-injection resulted in minimal local hair loss but induced a systemic reaction resulting in multiple extensive patches (McElwee et al. 2005). Immunoregulatory CD4+/CD25+ cells (immunosuppressive to CD4+/CD25- and CD8+ function) were found to decrease in active AA and prior to AA onset (Zoller et al. 2002).

AA has findings which extend beyond the affected sites. There is elevation of autoantibodies in serum, in addition to those found on lesional skin of patients with AA (Tobin 2003); however, the transfer of serum or autoantibodies has failed to induce alopecic patches (Gilhar et al. 1992); thus, these antibodies do not seem to have a primary role in the pathogenesis of AA.

The normal hair follicle is considered a relatively immune-privileged site through the downregulation of major histocompatibility complex (MHC) molecules (needed for autoantigen presentation to CD8+ T cells) and expression of immunosuppressive cytokines. In AA, an aberration in immune privilege in susceptible individuals may contribute to its pathogenesis. In the follicular epithelium of AA patients, there is an increase in MHC I and II expression and lower levels of immunosuppressive cytokines in contrast to non-AA hair follicles (Gilhar 2010; Gilhar et al. 2012). The immune-inhibitory molecules produced by anagen hair follicles which were found to be decreased in AA were alpha-melanocyte-stimulating hormone (alpha-MSH), transforming growth factor beta (TGF beta), indoleamine 2,3 dioxygenase (IDO), and Red/IK (Gilhar 2010). The decreased Red/IK cytokine in the outer root sheath of the hair follicle and hair matrix functions to suppress MHC II antigen (expression of which is triggered by IFN gamma) associated with autoimmune disease (Kang et al. 2012). There are elevated numbers of NKG2D+NK cells around lesional hair follicles in AA, and an agonist of NKG2D, MHC I polypeptide sequence A (MICA), is expressed in AA (Ito et al. 2008).

Many patients have positive history of the disease in genetically related family members.

Support for AA genetic susceptibility is being provided by study findings of genes relating to the immune system and regulation of the hair follicle.

A genome-wide association study (n = 1,054 AA cases; n = 3,278 controls) done in North America looking into the genetic component of alopecia areata has identified genes involved in autoimmunity and cell-mediated immunity specifically susceptibility loci on chromosomes 2q33.2(CTLA4), 4q27(IL2/IL21), 6P21.32(HLA), 6q25.1 (ULBP6/ULBP3), 9q31.1 (STX17), 10p15.1(IL2RA), 11q13 (PRDX5), and 12q13 (Eos/ERBB3) (Petukhova et al. 2010).

Of the aforementioned genes, the cytotoxic T-lymphocyte-associated antigen (CTLA4), interleukin (IL)2/IL21, and IL2 receptor A (IL2RA) have T cell proliferation function. Human leukocyte antigen (HLA) is involved with antigen presentation. Cytomegalovirus UL16-binding protein consisting of ULBP6/ULBP3 genes encoding activating ligands of the natural killer cell receptor NKG2D is being expressed in the hair follicle particularly in the dermal sheath of patients with AA. Lymphocytes around hair follicles composed of CD8+ can be activated by NKG2D-activating ligand. Two hair follicle genes were identified: syntaxin (STX17) and peroxiredoxin (PRDX5) (Petukhova et al. 2010).

A follow-up genome-wide association study in Europe (n = 1,702 cases; n = 1,723 controls) confirmed the significant association with the susceptibility loci for ULBP6/ULBP3, STX17, IL2/IL21, PRDX5, and ERBB3. In addition, two other susceptibility loci were identified: IL-13 produced by T helper cells with inflammatory cell recruitment role and KIA0359/CLEC16A expressed by immune cells but whose function is not yet known (Jagielska et al. 2012).

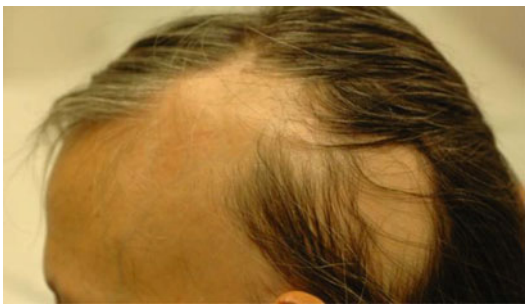
Some of the identified susceptibility alleles have also been found in other autoimmune diseases such as rheumatoid arthritis, type 1 diabetes mellitus, celiac disease, Crohn's disease, systemic lupus erythematosus, multiple sclerosis, and psoriasis signifying common autoimmune pathways (Petukhova et al. 2010).

Clinical Manifestations

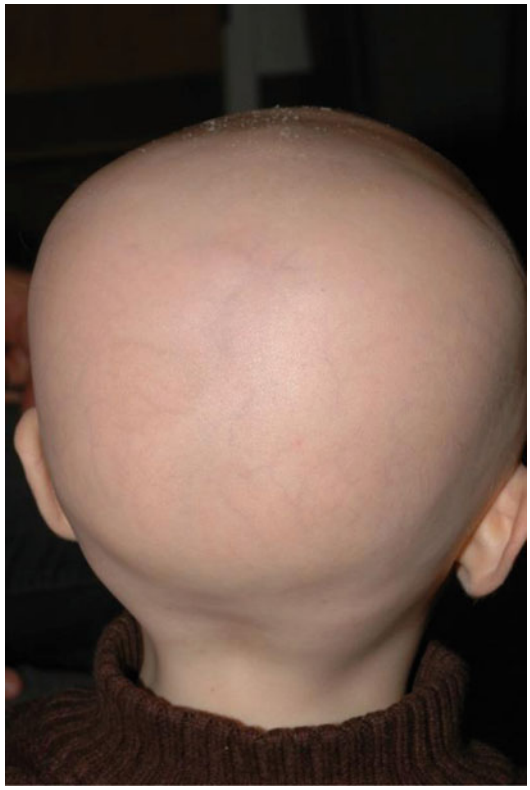
Clinically, AA presents as non-scarring, well-defined alopecic patches mainly found over the scalp in majority of cases. Other sites which may be affected include the eyebrows, eyelashes, beard, and body hair. The lesions typically start as discrete, solitary, or multiple patches (Fig. 1) which may remain limited in size or may expand to involve larger areas. The cutaneous surface of the affected patches are normal and smooth in appearance, with skin colored or pinkish hue. Upon closer examination, there is note of intact follicular ostia. Typically this condition occurs rapidly and there is variability in terms of disease severity. In severe progression of the disease, there may be hair loss of the entire scalp (AA totalis/AT) (Fig. 2) or body hair (AA universalis/AU) (Freyschmidt-Paul et al. 2008, 2010; Shapiro 2002).

Clinical variants based on pattern distribution include patchy, ophiasis, and diffuse type of AA. Patchy AA is the most common type affecting majority of cases. The ophiasis type is a band-like pattern which commonly starts at the occipital hairline area and extends towards the lateral temporoparietal hair margins. In diffuse AA, there are no well-circumscribed alopecic lesions but rather diffuse thinning areas over the scalp. This type is difficult to diagnose clinically and may need a biopsy for confirmation (Shapiro 2002).

AA may preferentially attack pigmented hairs leaving white hairs intact, resulting in the appearance of scalp hair suddenly turning white.



Alopecia Areata, Fig. 1 Patchy alopecia areata (Courtesy of the University of British Columbia Hair Clinic)



Alopecia Areata, Fig. 2 Alopecia areata totalis (Courtesy of the University of British Columbia Hair Clinic)

Positive pull test over the peripheral hair margins indicates active disease. Some lesions may have exclamation point hairs which appear as short broken hair fibers tapered proximally compared to its distal end.

Nails may also be affected with development of changes such as pitting, red lunulae, and longitudinal ridging. In some cases, nail signs may precede the appearance of the alopecic patches (Freyschmidt-Paul et al. 2010; Shapiro 2002).

Pathobiology and Histopathology

In the early stage of AA, there is a dense inflammatory infiltrate over the peribulbar region of the terminal anagen hair follicle (swarm of bees). Some infiltration may occur intrafollicularly as well (Whiting 2003; Dy and Whiting 2011).

Predominant T cells mainly CD4 and CD8 cause destruction (apoptosis and necrosis) to the follicular epithelium. This active inflammation leads to a dystrophic anagen condition wherein the affected hair follicle's caliber/quality becomes compromised. Upon progression of the disease with increased inflammatory cell infiltrates attacking the hair follicle, these affected follicles undergo a miniaturization process with shortened rapid cycling of the anagen and telogen phases (nanogen hair follicles) (Wang and McElwee 2011).

Inflammation causes disruption of the hair follicle, leading to disease activity. The inflammatory infiltrates are found mainly over the lower part of the hair follicle (hair matrix and dermal papilla), not the bulge area. Damage to the hair shaft results in trichorrhexis nodosa-like fractures seen clinically as exclamation point hairs. There may be injury to melanocytes, leading to pigment incontinence. Repeated inflammation leads to development of miniaturized hair follicles. Terminal hairs become less and catagen/telogen hair follicles rise in number, leading to the subsequent decrease of the anagen to telogen ratio (Whiting 2003; Dy 2011).

Chronic AA has hair follicles in prolonged telogen without reentry into the anagen phase. At this stage any inflammation present would be found surrounding miniaturized hairs. There is significant increase of miniaturized hairs and decrease to absence of terminal hairs. The telogen to vellus ratio may drop to 1:3 compared to the usual normal of 7:1 (Dy and Whiting 2011).

Treatment

To date, there is no standard curative and preventive treatment for AA. There is a high rate of spontaneous hair regrowth for localized patchy scalp disease; thus, not instituting any medical intervention is one management option.

Evaluating medications according to the evidence-based medicine criteria shows that few medications show effectivity in the treatment of AA. Among the immunosuppressive agents, potent topical and intralesional corticosteroids are reasonable first-line therapies for patients with limited patchy disease. Based on study results, the most effective topical corticosteroid

seems to be the superpotent clobetasol propionate foam for patchy AA and the 0.05 % ointment under occlusion for more extensive disease (Tosti et al. 2003, 2006).

Various clinical studies have shown efficacy of intralesional glucocorticoid ranging from 63 to 97 % (Alkhalifa 2011).

The dosage used for injections such as triamcinolone acetonide ranges from 2.5 to 10 mg/cc every 4–6 weeks.

Uncontrolled clinical trials on the pulsed administration of systemic corticosteroids have shown some efficacy; however, hair regrowth was mostly temporary with hair loss ensuing after discontinuation of treatment (Freyschmidt-Paul et al. 2010).

Many institutions consider topical immunotherapy as first-line treatment for more extensive lesions of AT and AU. The agents utilized for this treatment are Diphenylcyclopropenone (DPCP) and squaric acid dibutylester (SADBE). Various clinical trials including well-designed controlled studies on the effectivity of DPCP and SADBE showed response to treatment ranging from 29 to 78 % with a median of 49 % (Freyschmidt-Paul et al. 2010; Shapiro 2002).

The use of biologics (i.e., TNF alpha inhibitor (etanercept); LFA 3 inhibitor (alefacept)) so far has proven ineffective, and in some studies, TNF alpha inhibitors have even worsened the condition (Freyschmidt-Paul et al. 2010; Strober et al. 2009).

Other therapies such as topical minoxidil, anthralin, and phototherapy have not been proven effective in various clinical trials (Freyschmidt-Paul et al. 2010; Strober et al. 2009). Minoxidil, if used, is more of an adjunctive medication in addition to other treatments such as topical and intralesional corticosteroids.

Course and Prognosis

The lesions of AA are non-scarring with hair regrowth being possible. The course is characterized as unpredictable with disease recurrence occurring at any given time.

Within a year, there may be spontaneous hair regrowth seen in about half of the patients with localized patchy type of AA. There is however a high rate of recurrence within 5 years (Freyschmidt-Paul et al. 2010; Shapiro 2002).

Severe cases usually follow a chronic course. Factors associated with poor prognosis include severe type of AA (AT and AU), early age of onset, long-standing disease, presence of nail changes, and positive family history of AA and personal medical history of atopy and other autoimmune diseases (Shapiro 2002).

Cross-References

- ▶ Cytotoxic T Lymphocytes
- ▶ Immunology of Alopecia in Autoimmune Skin Disease
- ▶ Psoriasis
- ▶ Rheumatoid Arthritis, Genetics
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Alkalifah A, Alsantali A, Wang E, McElwee K, Shapiro J. Alopecia areata update: part I. Clinical picture, histopathology and pathogenesis. J Am Acad Dermatol. 2010;62:177–88.

Alkhalifa A. Topical and intralesional therapies for alopecia areata. Dermatol Ther. 2011;24:355–63.

Arca E, Musabak U, Akar A, Erbil AH, Tastan HB. Interferon gamma in alopecia areata. Eur J Dermatol. 2004;14(1):33–6.

Bodemer C, Peuchmar M, Fraitag S, Chatenaud L, Brousse N, De Prost Y. Role of cytotoxic T cells in chronic alopecia areata. J Invest Dermatol. 2000;114:112–6.

Carroll JM, McElwee K, King L, Byrne MC, Sundberg J. Gene array profiling and immunomodulation studies define a cell mediated immune response underlying the pathogenesis of alopecia areata in a mouse model and humans. J Invest Dermatol. 2002;119:392–402.

Dy L, Whiting D. Histopathology of alopecia areata, acute and chronic: why is it important to the clinician? Dermatol Ther. 2011;24:369–74.

Freyschmidt-Paul P, McElwee K, Hoffman R, et al. Interferon gamma deficient mice are resistant to the development of AA. Br J Dermatol. 2006;155:515–21.

Freyschmidt-Paul P, Hoffman R, McElwee K. Alopecia areata. In: Blume-Peytavi U, Tosti A, Whiting D, Trueb R, editors. Hair growth and disorders. Germany: Springer; 2008.

Freyschmidt-Paul P, McElwee K, Hoffman R. Alopecia areata. In: Hertl M, editor. Autoimmune diseases of the skin: pathogenesis, diagnosis, management. New York: Springer; 2012.

Gilhar A. Collapse of immune privilege in alopecia areata: coincidental or substantial? J Invest Dermatol. 2010;130:2535–7.
Gilhar A, Pillar T, Assy B, David M. Failure of passive transfer of serum from patients with alopecia areata and alopecia universalis to inhibit hair growth in transplants of human scalp skin grafted onto nude mice. Br J Dermatol. 1992;126:166–71.
Gilhar A, Etzioni A, Paus R. Alopecia areata. N Engl Med J. 2012;16:1515–25.
Ito T, Ito N, Saathoff M, et al. Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. J Invest Dermatol. 2008;128:1196–206.
Jagielska D, Redler L, Brockschmidt F, et al. Follow up of the first genome wide association scan in alopecia areata: IL 13 and KIAA0350 as susceptibility loci supported with genome wide significance. J Invest Dermatol. 26 April 2012. doi: 10.1038/jid.1012.129 (advance online publication).
Kang H, Wu WY, Lo BK, et al. Hair follicles form alopecia areata patients exhibit alterations in immune privilege-associated gene expression in advance of hair loss. J invest Dermatol Nov. 2012;130(11):2677–80.
Kurtev A, Iliev E. Thyroid autoimmunity in children and adolescents with alopecia areata. Int J Dermatol. 2005;44:457–61.
McElwee K, Hoffman R. Alopecia areata – animal models. Clin Exp Dermatol. 2002;27:414–21.
McElwee K, Hoffman R, Freyschmidt-Paul P, et al. Resistance to alopecia areata in C3H/HeJ mice is associated with increased expression of regulatory cytokines and a failure to recruit CD4+ and CD8+ cells. J Invest Dermatol. 2002;119:1426–33.
McElwee K, Freyschmidt-Paul P, Hoffman R, et al. Transfer of CD8+ cells induce localized hair loss while CD4+/CD25-cells promote systemic alopecia areata and CD4+/CD25+ cells blockade disease onset in the C3H/HeJ mouse model. J Invest Dermatol. 2005;124:947–57.
Petukhova L, Duvic M, Hordinsky M, et al. Genome wide association study in alopecia areata implicates both innate and adaptive immunity. Nature. 2010;466:113–7.
Safavi K. Prevalence of alopecia areata in the first national health and nutrition examination survey. Arch Dermatol. 1992;128:702.
Shapiro J. Alopecia Areata. In: Shapiro J, editor. Hair loss: principles of diagnosis and management of alopecia. London: Martin Duniz; 2002.
Strober B, Menon K, McMichael A, et al. Alefacept for severe alopecia areata. Arch Dermatol. 2009;145:1262–6.
Tobin DJ. Characterization of hair follicles antigens targeted by the anti-hair follicle immune response. J Invest Dermatol Symp proc. 2003;8:176–81.
Tosti A, Piraccine B, Pazzaglia M, Vincenzi C. Clobetasol propionate 0.05 % under occlusion in the treatment of alopecia totalis/universalis. J Am Acad. 2003;49:96–8.
Tosti A, Iorizzo M, Botta G, Milani M. Efficacy and safety of clobetasol propionate 0.05% foam in alopecia areata: a randomized, double blind, placebo controlled trial. J Eur Acad Dermatol Venereol. 2006;20:1243–7.
Wang E, McElwee K. Etiopathogenesis of alopecia areata: why do our patients get it? Dermatol Ther. 2011;24:337–47.
Wang SJ, Shohat T, Vadheim C, Shellow W, Edwards J, Rotter J. Increased risk for type I (insulin-dependent) diabetes in relatives of patients with alopecia areata. Am J Med Genet. 1994;51:234–9.
Whiting D. Histopathologic features of alopecia areata. Arch Dermatol. 2003;139:1555–9.

Alopecia in Systemic Autoimmune Disease

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Synonyms

Autoimmune disease and hair loss

Definition

Hair loss that is directly associated with autoimmune disease involves immune mechanisms in its pathogenesis targeting the hair follicles. They may be localized or generalized, as well as scarring and non-scarring in type. Secondary factors may also play a role in hair growth, including the medications used to treat the primary disease.

Lupus

Hair loss is one of the most common cutaneous signs of systemic lupus erythematosus (SLE)

and is present in more than half the patients at some time during the course of their illness (Moghadam-Kia and Franks 2013). Alopecia can be the presenting manifestation of SLE and may affect the scalp, eyebrows, eyelashes, beard hair, or body hair (McCauliffe and Sontheimer 1996). Alopecia may be associated with active disease and can also occur due to immunosuppressive medications used to treat lupus. Lupus alopecia can be scarring associated with discoid lupus or non-scarring. The Systemic Lupus International Collaborating Clinics (SLICC) has recently revised the American College of Rheumatology (ACR) SLE classification criteria (Petri et al. 2012). Discoid lupus has been always included in the most widely used classification criteria for SLE, including the new SLICC classification criteria. Non-scarring alopecia is also included in the new criteria as an individual criterion for SLE, as it was in the original ARA criteria.

Non-scarring Alopecia

Non-scarring alopecia in lupus often develops during flares of systemic disease and can occur under two scenarios. One is "telogen effluvium" that is a reactive process characterized by a diffuse thinning of the scalp and hair shedding caused by a metabolic or hormonal stress, like pregnancy, or the use of glucocorticoids. Generally, the normal hair usually grows back within 6 months when the antecedent etiology resolves. In diffuse patterned hair loss in general, up to 20 % of the hair can be lost before it is cosmetically visible. Therefore, complaints of hair loss should not be discredited in individuals who still have a full head of hair. Other scenario is "lupus hair," which is characterized by dry, course hair prominently over the frontal hairline that is very brittle and easily damaged. This condition is almost always observed during exacerbations of SLE and resolves when the disease activity abates (Alarcon-Segovia and Cetina 1974). Considerable overlap occurs between lupus hair and telogen effluvium. As in alopecia areata, non-scarring alopecia in lupus can occur as a wavy band-like area of hair loss extending across the peripheral temporal and occipital scalp (ophiasis pattern). Ophiasis gets its name from the Greek word "ophis," meaning snake.

Differential Diagnosis

Non-scarring alopecia in lupus must be distinguished from many other non-scarring alopecias. The differential diagnosis includes alopecia areata, telogen effluvium, traumatic alopecia, traction alopecia, female pattern hair loss, and male pattern alopecia (Table 2).

Alopecia Areata: Alopecia areata is a chronic inflammatory disease that causes recurrent non-scarring alopecia. It is thought to be an autoimmune condition caused by T cell-mediated immune dysfunction. There is an increased incidence of alopecia areata in patients with other autoimmune conditions such as lupus and DLE (Werth et al. 1992). It usually affects the scalp and presents with discrete circular well-delineated smooth patches of complete hair loss with no or little inflammation. The scalp lesions may be associated with burning sensation and slight erythema. Dermoscopy or 7x loupe magnification can aid in diagnosis and can reveal yellow dots and exclamation-point hairs with a tapering base and a ragged proximal portion that are diagnostic of the disease. Hairs that grow back are temporarily or permanently hypopigmented. This is another characteristic feature of alopecia areata. Scalp biopsy is useful is equivocal cases (Alkhalifah et al. 2010a, b).

Telogen Effluvium: Telogen effluvium is a non-scarring alopecia in which a physiologic stressor causes an increased number of hairs to go into resting phase (telogen phase). The hair loss in telogen effluvium occurs in a diffuse pattern. A hair-pull test can result in many hairs (more than 2 hairs on a group of 50 hairs) coming out easily from their roots with an elongated hair bulb. The scalp appears non-erythematous and unremarkable. Metabolic testing, including complete blood count, iron studies, thyroid function tests, serum chemistries, and liver enzymes, should be considered in the search for systemic causes of telogen effluvium. A drug history should be obtained as well. Scalp biopsy is usually not necessary (Tosti and Pazzaglia 2007).

Alopecia in Systemic Autoimmune Disease, Table 1 Comparative features of alopecia in lupus, dermatomyositis, and scleroderma

	Lupus	Dermatomyositis	Scleroderma
Frequency of scalp lesions	Frequently seen	Frequently seen	Occasionally seen
Frequency of alopecia	Frequently seen	Occasionally seen	Occasionally seen
Color of scalp lesions	Red, pink	Violaceous	Shiny, hypo- or hyperpigmented
Distinctive features of skin involvement	Involvement of the malar eminence, oral mucosa, dorsal fingers; hyperkeratosis	Involvement of the eyelids, periorbital areas, knuckles; pruritus	Systemic sclerosis (SSc): involvement of the fingers, hands, and face; edema and pruritus in the early stages; variable skin thickening; paucity of facial wrinkles; sclerodactyly; digital ulcers, calcinosis cutis; pitting at the finger tips; Raynaud's phenomenon
			Localized scleroderma: no Raynaud's phenomenon, no systemic involvement, no sclerodactyly; plaques tend to progress for 3–5 years and then arrest
Lab findings	Anti-DS DNA, Anti Smith	Autoantibodies directed against cytoplasmic RNA synthetases (Anti-Jo-1, Anti-PL-7, Anti-PL-12, Anti-OJ, Anti-EJ)	Limited cutaneous SSc: anticentromere antibody; diffuse cutaneous SSc: anti-Scl-70 (anti-topoisomerase I)
			Morphea: anti-histone antibodies
Pathology	Scalp DLE: intense mononuclear cell infiltrate, fibrosis, follicular hyperkeratosis, epidermal atrophy, thickened basement membrane, and basal vacuolar degeneration	Sparse perivascular lymphoid infiltrate, epidermal atrophy, basement membrane degeneration, and vacuolar changes in the basal keratinocyte layer	Intense inflammatory infiltrate at the margin at early stages and waning inflammatory infiltrate at later stages with infiltration of lymphocytes and plasma cells at the border and central fibrosis in the lower two-thirds of the dermis and upper subcutaneous tissue and eventually disappearance of pilosebaceous units and eccrine sweat glands and effacement of the rete ridges
Immunopathology	Dermal-epidermal junction granular deposits of immunoglobulin (IgG [less commonly IgM]) and complement (C3)	Dermal microvessel complement (C5 to C9) deposits (in DM, immunoglobulin deposition as opposed to complement is less common than in lupus)	Diffuse vascular deposits of immunoglobulins (predominantly IgM) and/or complement (predominantly Clq)
Associated comorbidities	SLE affects multiple organ systems	Up to 40 % of cases may be associated with occult malignancy	Epileptic seizures have been reported with en coup de sabre

Alopecia in Systemic Autoimmune Disease, Table 2 Differential diagnosis of non-scarring alopecia in lupus

Alopecia areata	Look for discrete circular well-delineated smooth patches of complete hair loss. Hairs that grow back are temporarily or permanently hypopigmented. Exclamation-point hairs with a tapering base and a ragged proximal portion that can be seen under dermoscopy are diagnostic of the disease
Telogen effluvium	Look for the history of a stressor or new medication, diffuse pattern of hair loss, and unremarkable examination of the scalp. A positive hair test with elongated hair bulbs is suggestive
Traction alopecia	Look for the history of long-term friction. The location of the hair loss at the temporal and frontal margins of the scalp, with broken fine vellus hairs at different stages, is suggestive
Traumatic alopecia (other than traction alopecia)	Trichotillomania: look for patches of alopecia with angulated and irregular borders and hairs that are broken off at varying lengths
Female pattern hair loss (FPHL)	Look for progressive thinning of hair in the central portion of the scalp with hairs of various lengths and diameter and retention of the frontal hair line. The loss is gradual. A family history is suggestive
Male pattern alopecia (MPA)	Look for progressive thinning of hair in an M-shaped pattern in the frontal hairline with bitemporal recession. The hair loss is gradual. A family history is suggestive

Traction Alopecia: Traction alopecia is a form of traumatic alopecia that occurs as a result of chronic and excessive tension on the hair follicles. Severe cases of traction alopecia due to chronic long-term friction can be associated with follicular atrophy and permanent non-scarring alopecia. Obtaining thorough history including the exact styling techniques and products used is necessary. The location of the hair loss at the temporal and frontal margins of the scalp, with broken fine vellus hairs at different stages, is a distinctive clinical feature of traction alopecia. The scalp appears normal without evidence of scarring. Scalp biopsy can be helpful in some cases that are hard to differentiate clinically (Borovicka et al. 2009).

Traumatic Alopecia (Other Than Traction Alopecia): These types of hair loss include trichotillomania and alopecia secondary to physical abuse. Trichotillomania usually occurs in adolescents during times of psychosocial stress. It usually presents with patches of alopecia with angulated and irregular borders and with broken hairs of different lengths, usually located on the frontotemporal or frontoparietal scalp opposite the dominant hand. Affected areas are not completely bald. Localized perifollicular erythema or hemorrhage may occur. Scalp biopsy can be helpful in complicated cases. Alopecia due to physical abuse can be difficult to differentiate from other types of alopecia. If there is concern for abuse, look for historical inconsistencies, signs of trauma such as scalp hematoma or tenderness, and psychosocial risk factors. Signs of inflammation are absent in all types of traumatic alopecia.

Female Pattern Hair Loss (FPHL): FPHL, also referred to as female androgenetic alopecia, is the most common type of hair loss in adult women. FPHL usually presents with diffuse thinning of the central portion of the scalp with retention of the frontal hair line. The affected area is usually widened and more obvious. Bitemporal recession is rare. The hair loss in FPHL is gradual with conversion of pigmented thick terminal hairs to shorter indeterminate hairs and finally nonpigmented miniaturized vellus hairs. These hairs of various lengths and diameter are classic signs of androgenetic alopecia. A family history of similar hair loss is suggestive of FPHL. A hair-pull test may be helpful (3 or fewer hairs on a

group of 20 hairs indicating normal shedding). Follicles are intact. Scalp biopsy may help rule out autoimmune or inflammatory disorders (Olsen et al. 2005).

Male Pattern Alopecia (MPA): MPA, or androgenetic alopecia, is the most common type of alopecia in adult men. It often affects men prior to the age of 40. MPA usually presents with progressive thinning of hair in an M-shaped pattern in the frontal hairline with bitemporal recession that moves posteriorly as the alopecia progresses. Similar to FPHL, the hair loss in MPA is gradual with hairs undergoing a transition from terminal hairs to indeterminate hairs to vellus hairs. Follicles are intact without evidence of scarring. Taking a family history can be helpful (Olsen et al. 2005; Sinclair 1998).

Scarring Alopecia

Introduction

Scarring alopecia or cicatricial alopecia associated with discoid lupus is categorized as an LE-specific skin disease on the Gilliam's classification (Gilliam and Sontheimer 1981, 1982).

Epidemiology and Pathogenesis

Scarring alopecia is a frequent complication of discoid lupus erythematosus (DLE, a form of chronic cutaneous lupus erythematosus according to the Gilliam's classification (Ross et al. 2005)) and has been reported in more than half (34–56 %) of the patients with DLE. Scalp DLE is present in 4–14 % of patients with SLE (Yell et al. 1996). Scalp DLE can be the presenting manifestation of lupus in more than half of affected individuals and can remain the only manifestation of disease in 11–20 %. It has been shown to correlate with disease chronicity. Scalp DLE affects females more often than male (Whiting 2001; Tan et al. 2004). Onset of disease is usually between 20 and 30 years of age (Tan et al. 2004 p. 30). Onset of disease has been reported to occur less frequently in children and particularly those under age 10 (Moises-Alfaro et al. 2003).

The action of genetic, environmental, immunoregulatory, hormonal, and epigenetic factors involved in the pathogenesis of lupus results in the generation of inflammatory T cells, inflammatory cytokines, autoantibodies, and immune complexes that may cause damage to various target organs. Progressive replacement of the follicular epithelium by connective tissue and varying degree of permanent injury to the pluripotent hair follicle stem cell region in the bulge of hair follicles (where the arrector pili muscle connects to the outer root sheath) is similar to other forms of scarring alopecia. Like other variants of cicatricial alopecia, permanent destruction of hair follicles in DLE is frequently associated with a loss of sebaceous gland (Sellheyer and Bergfeld 2006). In scalp DLE, the localization of inflammation around the upper, permanent portion of the hair follicle appears to result from antigenic stimulation of the Langerhans cells that are positioned in the follicular epithelium below the entry of sebaceous glands into the follicle. These Langerhans cells may then trigger a first-line T cell- or immune complex-mediated inflammatory response (Dutz and Sontheimer 2002). This pattern of follicular inflammation is similar to scarring folliculitides of lichen planopilaris, allogeneic graft-versus-host reaction, and atopy. In scalp DLE, the antigenic stimulus affecting the Langerhans cells appears to be ultraviolet light (Ross et al. 2005); however, its role on hair-bearing scalp, a relatively sun-protected site, needs further study. A study showed that patients with coexisting androgenetic alopecia do not preferentially develop DLE in bald areas. The Koebner phenomenon is associated with DLE. Constant rubbing and scratching can lead to new lesions in affected patients. The proinflammatory cytokines interleukin-17 (IL-17), interleukin-23 (IL-23), and IL-17-producing cells have been shown to be important in the pathogenesis of lupus and lupus nephritis (Crispín et al. 2008; Zhang et al. 2009). A recent study of 89 patients with systemic and cutaneous lupus showed that IL17 isoforms (IL-17A and IL-17 F) are implicated not only in SLE but also in DLE immunopathogenesis (Tanasescu et al. 2010). Another recent study on 15 subjects with lupus suggested that T helper 17 lymphocytes and IL-17 are involved in the immunopathogenesis of both SLE and DLE.

As previously mentioned, DLE scarring alopecia is considered a primary scarring alopecia as the target of inflammation seems to be the hair follicle. For primary cicatricial alopecia, several classification systems exist in the literature; however, it is still controversial. In 2001, the North American Hair Research Society (NAHRS) developed a provisional classification for primary cicatricial alopecia. This classification scheme is a mechanistic classification system based on pathologic interpretation of dominant inflammatory cell type existing in and around affected hair follicles in scalp biopsy taken from clinically active lesions. This scheme divides the entities into lymphocytic, neutrophilic, mixed, or unspecific. Alopecia due to DLE is considered to be the most common primary acquired lymphocytic scarring alopecia (Tan et al. 2004).

Clinical Manifestations

Early classic lesion presents as a quite well-circumscribed, erythematous, infiltrative patch with adherent follicular hyperkeratosis. Later, the lesion progresses centrifugally to form a coin-shaped ("discoid") white-ivory, atrophic, depressed, smooth plaque with follicular plugging and adherent scale. Telangiectasia might also be present (Whiting 2001; Shapiro 2002; Fabbri et al. 2004; Donnelly et al. 1995). They may have features of classic discoid lesions elsewhere. In darker-skinned individuals central hypopigmentation and peripheral hyperpigmentation may occur (Sontheimer and McCauliffe 2002). The scalp lesions may resemble alopecia areata, lichen planopilaris, or linear morphea. Discoid lesions of lupus in the scalp can commonly be pruritic or tender; however, the condition may be asymptomatic. The patients might report that UV exposure worsens their symptoms.

Diagnosis

The initial approach to the patient with scalp DLE should include examination of the entire scalp, assessing the location and pattern of hair loss and also the presence of extracranial cutaneous and systemic features. Scalp biopsy with adjunctive use of direct immunofluorescence is helpful in establishing the diagnosis and evaluating the degree of inflammation and differentiation of scalp DLE from other primary lymphocytic cicatricial alopecias, respectively. Scalp biopsy specimens should be from early clinically active disease, at least 4 mm in diameter, and extend into the fat. Ideally, two biopsy specimens, one for standard horizontal sections and one for longitudinal section, should be obtained for transverse and vertical suctioning (Shapiro 2002). The major histopathologic features include fibrosis, follicular hyperkeratosis, epidermal atrophy, lymphocytic infiltrate, thickened basement membrane, and basal vacuolar degeneration. Granular deposits of C3 and IgG (less commonly IgM) at the dermal-epidermal junction and/or the junction of the follicular epithelium and dermis are typical. These histopathologic aspects can resemble those found in lichen planopilaris, an inflammatory scarring alopecia (Fabbri et al. 2004).

Differential Diagnosis

DLE scarring alopecia must be distinguished from other conditions that cause alopecia. The differential diagnosis of scalp DLE includes lichen planopilaris, radiation-induced alopecia, central centrifugal cicatricial alopecia, sarcoidosis, psoriasis, burn scar, and squamous cell carcinoma. In addition, a variety of other scalp conditions such as tinea capitis can be very inflammatory and must also be considered. Less commonly, non-scarring alopecia can be confused for scalp DLE (Table 3).

Lichen Planopilaris (LPP): LPP, also known as follicular lichen plan, can cause scarring alopecia over time. As in lichen planus, LPP is an autoimmune condition that is most likely caused by cell-mediated immune dysfunction. Similar to the pattern of follicular inflammation in scalp DLE, T lymphocytes targeted at follicular antigens are involved. LPP occurs more frequently in women than men. Patient with lighter skin are more commonly affected than dark-skinned individuals. There are three variants of LPP: classic LPP, frontal fibrosing alopecia, and Graham-Little syndrome. Classic LPP is characterized by perifollicular erythema and patches of alopecia

Alopecia in Systemic Autoimmune Disease, Table 3 Differential diagnosis of scarring alopecia associated with discoid lupus

Lichen planopilaris (LPP)	Look for erythema that is confined to perifollicular areas (in contrast to scalp DLE) and keratotic plugs surrounding the patches of alopecia. Loss of follicular orifices can be viewed under dermoscopy. Dyspigmentation, in comparison with scalp DLE, is less common
Central centrifugal cicatricial alopecia (CCCA)	Look for shiny scarring alopecia usually seen from the vertex forward. The presence of burning sensation or pruritus in the area of hair loss can help. Premature desquamation of the inner root sheath in scalp biopsy is suggestive
Radiation-induced alopecia	Look for the history of radiation exposure and regular and sharp borders. Decreased number of follicular units with fibrosis of adjacent collagen in scalp biopsy is suggestive
Squamous cell carcinoma	Look for long-standing hyperkeratotic or ulcerated lesions and scars. Biopsy is needed to confirm the diagnosis
Tinea capitis	Look for signs of inflammation in the scalp including erythema and scaling. Positive fungal culture and examination of plucked hairs with KOH are diagnostic

with surrounding keratotic plugs. Frontal fibrosing alopecia presents with band-like scarring alopecia of the frontal hairline that commonly affects women. Graham-Little syndrome is characterized by scarring alopecia of the scalp, non-scarring alopecia of the pubic and axillary areas, and a lichenoid follicular eruption. In contrast to scalp DLE, erythema is confined to perifollicular areas in LPP. Also, dyspigmentation is less commonly seen. Dermoscopy or 7x loupe magnification can aid in revealing the perifollicular erythema and loss of follicular orifices. A hair-pull test may reveal an increased number of anagen hairs. Scalp biopsy performed at the margin of alopecia from the most active area of disease is the most useful test for the diagnosis. Histologic features of LPP include a lichenoid interface inflammation around the infundibulum and isthmus, sparing the hair bulb. Hyperkeratosis, acanthosis, and hypergranulosis can also be seen. In advanced disease, significant perifollicular lamellar fibrosis can be seen. Direct immunofluorescence is nonspecific and may show colloid body staining with IgM. These histopathologic features can resemble those found in scalp DLE.

Central Centrifugal Cicatricial Alopecia: Formerly known as follicular degeneration syndrome, central centrifugal cicatricial alopecia (CCCA) is a slowly progressive scarring alopecia that usually occurs in black women. CCCA usually presents with increased follicular spacing and circle-shaped, shiny flesh-colored, smooth scarring alopecia. In contrast to scalp DLE, CCCA usually involves the crown or vertex and expands centrifugally. The presence of burning sensation or pruritus in the area of hair loss can also help to distinguish CCCA from other types of scarring alopecia. A characteristic histologic feature of CCCA on scalp biopsy is premature desquamation of the inner root sheath (Borovicka et al. 2009).

Radiation-Induced Alopecia: Radiation-induced alopecia commonly occurs after therapeutic radiation for head and neck cancers or inadvertent overdose. Low radiation dose will lead to reversible alopecia. Higher doses can result in severe erythema weeks after the radiation exposure followed by poikilodermatous changes and irreversible scarring alopecia. In contrast to scalp DLE, radiation-induced alopecia often has regular and sharp borders. Radiation-induced alopecia is localized to the treatment zone and the shape and pattern of the alopecia in relevant to the radiation delivery window. Histopathologic features of radiation-induced alopecia include decreased number of follicular units with fibrosis or hyalinization of adjacent collagen.

Squamous Cell Carcinoma: DLE lesions and particularly long-standing hyperkeratotic lesions and scars of chronic DLE are thought to be a predisposing factor for squamous cell carcinoma, with a high rate of local recurrence and metastasis. Close observation of every alopecic area is mandatory to determine ulcerated or hyperkeratotic lesions, all of which should be biopsied (Ross et al. 2005).

Tinea Capitis: Tinea capitis, a *Trichophyton tonsurans* infection of the scalp, is usually associated with signs of inflammation including erythema and scaling. Cervical adenopathy can be present. Positive fungal culture and examination of plucked hairs with KOH are diagnostic for tinea capitis.

Therapy and Prognosis

DLE scarring alopecia is usually irreversible and the inflammation affects the upper portion of the hair follicle including critical elements within the mid-follicle required for follicular reconstruction, as opposed to non-scarring alopecias such as alopecia areata that affect the lower portion of the hair follicle and wherein the follicle has the potential to regrow hair (Tan et al. 2004). DLE scarring alopecia can lead to considerable societal costs and reduced quality of life. A recent study by Ferraz et al. showed that lupus patients with alopecia had lower quality of life (Ferraz et al. 2006). In contrast, diffuse non-scarring alopecia in lupus is usually responsive to treatment of the lupus; however, it can be occasionally persistent, particularly in individuals with persistent active systemic disease (Moghadam-Kia and Franks 2013).

Dermatomyositis

Dermatomyositis (DM) is a systemic autoimmune connective tissue disease that is classified as an idiopathic inflammatory myopathy (Dalakas & Hohlfeld 2003). DM most frequently occurs between the age of 40 and 50, but it can affect any age group. There is a female predominance (female: male, 2:1) (Tymms and Webb 1985). DM is associated with hallmark skin findings including distinctive rashes (Gottron's sign and heliotrope rash) and poikiloderma (hypo- and hyperpigmentation, telangiectasis, and epidermal atrophy). Involvement of scalp, manifested as diffuse, confluent, atrophic, violaceous, scaly plaques, can be commonly seen in DM and can be the presenting manifestation of DM; however, alopecia is only occasionally seen. In a case series of 17 patients with DM, scalp involvement was noted in 14, with alopecia present in 6 of the 14 patients (Kasteler and Callen 1994). Adult-onset classic DM and clinically amyopathic DM can be associated with scaly scalp and non-scarring diffuse alopecia that often follows a flare of the systemic disease (Euwer and Sonthheimer 1996; Callen and Wortmann 2006; Callen 2000; Santmyire-Rosenberger and Dugan 2003). This diffuse violaceous scaly alopecia is one of the characteristic cutaneous features of DM, despite not being pathognomonic (Euwer and Sonthheimer 1996; Callen and Wortmann 2006). Non-scarring alopecia has also been reported in juvenile-onset DM. Also, DM may rarely cause cicatricial alopecia. DM may overlap with features of other connective tissue disease, particularly scleroderma and lupus (Dawkins et al. 1998). The clinical features that can help to distinguish DM from lupus include the violaceous color of the poikiloderma (in contrast to the red poikiloderma in lupus, similar to the violaceous hue in lichen planus) and localization of lesions around the eyes and on the extensor surfaces and severe pruritus in DM. Also, the scale in DM skin lesions is usually less prominent than in lupus. The histopathologic features include epidermal atrophy, basement membrane degeneration, vacuolar changes in the basal keratinocyte layer, and a sparse perivascular lymphoid infiltrate; the changes may be difficult to distinguish from those seen in lupus on light microscopy. Like in lupus, the dermis is often pale due to the accumulation of mucin and edema. Immunofluorescence microscopy reveals an interface dermatitis (deposition of complements and immunoglobulin

at the dermal-epidermal junction) (in DM, immunoglobulin deposition as opposed to complement is less common than in lupus) (Dourmishev and Wollina 2006). Muscle biopsy can be also performed. A combination of type 2 muscle fiber atrophy and lymphocytic infiltrate in both a perifascicular and a perivascular distribution is classic.

Scleroderma

Scleroderma is a systemic autoimmune connective tissue disease. It is more common in women and the peak age of onset is between 30 and 50 years. The disease involves the autoantibodies to characteristic cellular antigens and characteristic sclerotic changes of skin. Scleroderma is different from other autoimmune diseases involving skin (lupus, dermatomyositis) because epithelial injury does not occur (Gilliam 2008). The sclerotic changes can affect the connective tissue on any organ. When the disease is associated with internal organ involvement, it is named systemic sclerosis (SSc). There are two major subsets of SSc based on the degree of cutaneous involvement: limited cutaneous SSc and diffuse cutaneous SSc. SSc is characterized by typical cutaneous changes, including variable extent and severity of skin thickening, shiny and wrinkleless skin, diffuse hyperpigmentation, and depigmentation with sparing of perifollicular skin, leading to a salt-and-pepper appearance and flat telangiectasis. SSc usually affects the fingers, hands, and face. Autoantibodies including antinuclear antibodies (ANA) with a discrete speckled or nucleolar pattern, anti-Scl-70, and anti-RNP antibodies assist in the diagnosis (Gilliam 2008; Chung et al. 2006). Cyclophosphamide, the prototypic alkylating and immunosuppressant agent that has been used to treat systemic scleroderma, is associated with alopecia (3 %).

Localized form of scleroderma, also known as morphea, is a self-limited inflammatory disorder. Morphea is a relatively uncommon disorder that may occur at any age, but most frequently affects young adults and children. There is a female to male predominance of about 3:1, and the condition is uncommon in blacks. Like SSc, morphea is also characterized by spontaneous sclerosis of the skin but lacks internal organ involvement, Raynaud's phenomenon, and sclerodactyly. It involves transition from an early inflammatory stage to sclerosis and subsequent atrophy after 2–3 years. It usually presents with shiny, oval, 10 cm or greater in diameter, firm, indurated plaques with surrounding erythema. The surrounding red or violaceous rim may fade and transition into hypo- or hyperpigmentation with time. Hair follicles and sweat glands are absent in well-developed lesions. The lesions usually affect the trunk and extremities. The most common forms of morphea are plaque, generalized, and linear variants. A form of linear morphea that affects the face or scalp, usually the midline or paramedian forehead, is known as en coup de sabre because the lesion is reminiscent of a cut of a sword. It usually presents as a unilateral, shiny, hypo- or hyperpigmented, atrophic, linear plaque. It can present with more than one lesion, typically following Blashcko's lines, extend onto the scalp, and cause permanent cicatricial alopecia secondary to loss of hair follicles. The diagnosis is usually made clinically but can be confirmed with a skin biopsy. Biopsies at early stages reveal an intense inflammatory infiltrate at the margin, and biopsies at later stages reveal waning inflammatory infiltrate with infiltration of lymphocytes and plasma cells at the border and central fibrosis in the lower two-thirds of the dermis and upper subcutaneous tissue and eventually disappearance of pilosebaceous units and eccrine sweat glands and effacement of the rete ridges, similar to the changes seen in SSC. Ultrasound can be used to assess skin thickness which correlates with disease severity. Table 1 summarizes the comparative features of alopecia in lupus, dermatomyositis, and scleroderma.

Fibromyalgia

Fibromyalgia is thought to be a functional somatic syndrome caused by alterations in central nervous system's pain processing. It is characterized by chronic generalized musculoskeletal pain, fatigue, and multiple tender points at

Alopecia in Systemic Autoimmune Disease, Table 4 Alopecia due to medications used to treat systemic autoimmune disease and fibromyalgia[a]

Associated medications
Citalopram
Cyclophosphamide
Danazol
Fluoxetine
Fluvoxamine
Gold
IFN alpha
IVIG
Leflunomide
Methotrexate
Mycophenolate mofetil
Phenytoin
Tacrolimus
Venlafaxine

[a]Many drugs that are currently used to treat systemic autoimmune disease have been reported to cause alopecia. The medication-induced hair loss is usually diffuse and non-scarring. The hair loss is usually limited to the scalp. Women are more commonly affected than men

specific soft tissue locations. There is typically no evidence of joint or muscle inflammation on physical examination or laboratory testing. Fibromyalgia is currently considered to be the most common cause of widespread musculoskeletal pain in women between 20 and 55 years of age. The prevalence is approximately 2 % and increases with age. Fibromyalgia may coexist with other inflammatory rheumatic diseases, such as SLE which can cause non-scarring or scarring alopecia. The medications that are used in the treatment of fibromyalgia can also cause alopecia. Tricyclic antidepressants including amitriptyline and desipramine can be associated with alopecia. Serotonin reuptake inhibitors, particularly fluoxetine and citalopram, can rarely (<1 %) cause alopecia (Table 4).

Cross-References

- ▶ Alopecia Areata
- ▶ Hair Loss in Lupus Erythematosus

References

Alarcon-Segovia D, Cetina JA. Lupus hair. Am J Med Sci. 1974;267(4):241–2.

Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: part I. Clinical picture, histopathology, and pathogenesis. J Am Acad Dermatol. 2010a;62(2):177–88.

Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: part II. Treatment. J Am Acad Dermatol. 2010b;62(2):191–202.

Borovicka JH, Thomas L, Prince C, Mehregan DR. Scarring alopecia: clinical and pathologic study of 54 African-American women. Int J Dermatol. 2009;48(8):840–5.

Callen JP. Dermatomyositis. Lancet. 2000;355(9197):53–7.

Callen JP, Wortmann RL. Dermatomyositis. Clin Dermatol. 2006;24(5):363–73.

Chung L, Lin J, Furst DE, Fiorentino D. Systemic and localized scleroderma. Clin Dermatol. 2006;24(5):374–92.

Crispín JC, Oukka M, Bayliss G, Cohen RA, Van Beek CA, Stillman IE, Kyttaris VC, Juang YT, Tsokos GC. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. J Immunol. 2008;181(12):8761–6.

Dalakas MC, Hohlfeld R. Polymyositis and dermatomyositis. Lancet. 2003;362(9388):971–82.

Dawkins MA, Jorizzo JL, Walker FO, Albertson D, Sinal SH, Hinds A. Dermatomyositis: a dermatology-based case series. J Am Acad Dermatol. 1998;38(3):397–404.

Donnelly AM, Halbert AR, Rohr JB. Discoid lupus erythematosus. Australas J Dermatol. 1995;36(1):3–10; quiz 11–2. Review.

Dourmishev LA, Wollina U. Dermatomyositis: immunopathologic study of skin lesions. Acta Dermatovenerol Alp Panonica Adriat. 2006;15(1):45–51.

Dutz JP, Sontheimer RD. Pathomechanisms of cutaneous lupus erythematosus. In: Wallace DJ, Hahn BH, editors. Dubos' Lupus erythematosus. 6th ed. Philadelphia: Lippincot William & Wilkins; 2002. p. 549–71.

Euwer RL, Sonthheimer RD. Dermatomyositis. In: Sontheimer RD, Provost TT, editors. Cutaneous manifestations of rheumatic disease. Baltimore: Williams and Wilkins; 1996. p. 73.

Fabbri P, Amato L, Chiarini C, Moretti S, Massi D. Scarring alopecia in discoid lupus erythematosus: a clinical, histopathologic and immunopathologic study. Lupus. 2004;13(6):455–62.

Ferraz LB, Almeida FA, Vasconcellos MR, Faccina AS, Ciconelli RM, Ferraz MB. The impact of lupus erythematosus cutaneous on the Quality of life: the Brazilian-Portuguese version of DLQI. Qual Life Res. 2006;15(3):565–70.

Gilliam AC. Scleroderma. Curr Dir Autoimmun. 2008;10:258–79.

Gilliam JN, Sontheimer RD. Distinctive cutaneous subsets in the spectrum of lupus erythematosus. J Am Acad Dermatol. 1981;4(4):471–5.

Gilliam JN, Sontheimer RD. Skin manifestations of SLE. Clin Rheum Dis. 1982;8(1):207–18.
Kasteler JS, Callen JP. Scalp involvement in dermatomyositis. Often overlooked or misdiagnosed. JAMA. 1994;272(24):1939–41.
McCauliffe DP, Sontheimer RD. Cutaneous lupus erythematosus. In: Schur PH, editor. The clinical management of systemic lupus erythematosus. Secondth ed. Philadelphia: Lippincott; 1996.
Moghadam-Kia S, Franks Jr AG. Autoimmune disease and hair loss. Dermatol Clin. 2013;31(1):75–91.
Moises-Alfaro C, Berrón-Pérez R, Carrasco-Daza D, Gutiérrez-Castrellón P, Ruiz-Maldonado R. Discoid lupus erythematosus in children: clinical, histopathologic, and follow-up features in 27 cases. Pediatr Dermatol. 2003;20(2):103–7.
Olsen EA, Messenger AG, Shapiro J, et al. Evaluation and treatment of male and femalepattern hair loss. J Am Acad Dermatol. 2005;52(2):301–11.
Petri M, Orbai A, Alarcón G, et al. Derivation and validation of systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012;64(8):2677–86.
Ross EK, Tan E, Shapiro J. Update on primary cicatricial alopecias. J Am Acad Dermatol. 2005;53(1):1–37; quiz 38–40. Review. Erratum in: J Am Acad Dermatol. 2005;53(3):496.
Santmyire-Rosenberger B, Dugan EM. Skin involvement in dermatomyositis. Curr Opin Rheumatol. 2003;15(6):714–22.
Sellheyer K, Bergfeld WF. Histopathologic evaluation of alopecias. Am J Dermatopathol. 2006;28(3):236–59.
Shapiro J. Cicatricial (scarring) alopecias. In: Shapiro J, editor. Hair loss: principles of diagnosis and management of alopecia. London: Martin Duniz; 2002. p. 155–74.
Sinclair R. Male pattern androgenetic alopecia. BMJ. 1998;317(7162):865–9.
Sontheimer RD, McCauliffe DP. Cutaneous manifestations of lupus erythematosus. In: Wallace DJ, Hahn BH, editors. Dubos' lupus erythematosus. 6th ed. Philadelphia: Lippincot William & Wilkins; 2002. p. 573–618.
Tan E, Martinka M, Ball N, Shapiro J. Primary cicatricial alopecias: clinicopathology of 112 cases. J Am Acad Dermatol. 2004;50(1):25–32.
Tanasescu C, Balanescu E, Balanescu P, Olteanu R, Badea C, Grancea C, Vagu C, Bleotu C, Ardeleanu C, Georgescu A. IL-17 in cutaneous lupus erythematosus. Eur J Intern Med. 2010;21(3):202–7.
Tosti A, Pazzaglia M. Drug reactions affecting hair: diagnosis. Dermatol Clin. 2007;25(2):223–31.
Tymms KE, Webb J. Dermatopolymyositis and other connective tissue diseases: a review of 105 cases. J Rheumatol. 1985;12(6):1140–8.
Werth VP, White WL, Sanchez MR, Franks AG. Incidence of alopecia areata in lupus erythematosus. Arch Dermatol. 1992;128(3):368–71.
Whiting DA. Cicatricial alopecia: clinico-pathological findings and treatment. Clin Dermatol. 2001;19(2):211–25.
Yell JA, Mbuagbaw J, Burge SM. Cutaneous manifestations of systemic lupus erythematosus. Br J Dermatol. 1996;135(3):355–62.
Zhang Z, Kyttaris VC, Tsokos GC. The role of IL-23/IL-17 axis in lupus nephritis. J Immunol. 2009;183(5):3160–9.

Animal Models in Rheumatoid Arthritis

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Synonyms

Inflammatory arthritis animal model

Definition

Rheumatoid arthritis is the most common form of inflammatory joint disease and is characterized by synovial hyperplasia, immune cell infiltration, pannus formation, cartilage destruction, and bone erosion. A variety of animal models of arthritis have been established, including those that involve the injection of TNF-alpha, proteoglycan, adjuvant, antibody, or collagen. These animal models demonstrate synovitis, pannus formation, bone and cartilage destruction, as well as other features observed in human rheumatoid arthritis. They provide a useful platform to investigate the pathogenesis and determine the effects of novel therapies for rheumatoid arthritis.

Rheumatoid Arthritis

Rheumatoid arthritis is the most common form of inflammatory arthritis and is characterized by synovial hyperplasia, immune cell infiltration, pannus formation, cartilage destruction, and bone erosion (Feldmann and Maini 2003). In the inflamed synovial membrane, highly prevalent cells include T cells and macrophages, while fibroblasts, plasma cells, endothelium cells, and

dendritic cells are present in lesser numbers. In the synovial fluid, the predominant cell is the neutrophil. Bone destruction is a remarkable feature and caused by bone resorption increasing more than bone formation.

Although the etiology and pathogenesis of rheumatoid arthritis are not yet fully understood, cytokines such as TNF-alpha, interleukin (IL)-1, IL-6, and receptor activator of NF-KB ligand (RANKL) are known to be involved in disease progression. Evidence demonstrates that TNF-alpha plays a crucial role in the pathogenesis of rheumatoid arthritis. TNF-alpha can stimulate osteoclastogenesis (Deng et al. 2005) and osteoclasts are crucial cells in bone destruction. In animal models of RA, intra-articular injection of TNF-alpha can induce arthritis directly (1), TNF-alpha transgenic mice spontaneously develop arthritis in multiple joints, and anti-TNF treatment inhibits development of arthritis. Meanwhile, TNF-alpha-deficient mice are resistant to the development of arthritis. Thus, TNF-alpha is an important therapeutic target in rheumatoid arthritis. Currently, a number of anti-TNF medications are highly effective in the treatment of rheumatoid arthritis (Feldmann and Maini 2003).

To gain insight into the etiology, pathogenesis, and response to therapy, a variety of animal models of rheumatoid arthritis have been established.

Animal Models in Rheumatoid Arthritis

Collagen-Induced Arthritis

Collagen-induced arthritis (CIA) is the most commonly used animal model of rheumatoid arthritis (Deng et al. 2005). Arthritis is induced by immunization of mice, rats, and monkeys with various cartilage proteins after tolerance is broken and an immune/inflammatory response is directed against the joints. The most commonly used immunogen is type II collagen which is injected in DBA/1J mouse. Chicken collagen type II emulsified with an equal volume of complete Freund's adjuvant with 0.1 ml of emulsion containing 100 μg of type II collagen is intradermally injected at the base of the tail of male DBA/1J mouse. A booster injection of 100 μg of type II collagen emulsified with an equal volume of incomplete Freund's adjuvant is administered to the mouse 21 days after the first immunization. The extent of arthritis is determined by evaluating clinical and histological scores. CIA is a well-controlled and reproducible model for RA.

Clinical Evaluation of Arthritis: Joint swelling is determined by measuring the thickness of the paws with a caliper. Clinical arthritis is assessed using the following scale: grade 0, no swelling; grade 1, slight swelling and erythema; grade 2, pronounced swelling; and grade 3, joint rigidity. Each limb is graded with a score of 0–3, with a maximum possible score of 12 for each mouse.

Histological Examination of Arthritis: After routine fixation with 10 % formalin, decalcification, and paraffin embedding of the joint tissue, joint sections are cut and stained with hematoxylin and eosin. The extent of synovitis, pannus formation, or bone and cartilage destruction is evaluated. A scale is used as follows: grade 0 (no signs of inflammation), grade 1 (mild inflammation with minimal hyperplasia of the synovial lining layer without cartilage destruction), and grades 2 through 4 (increasing degrees of inflammatory cell infiltrate or cartilage and bone destruction).

Adjuvant-Induced Arthritis

Adjuvant-induced arthritis (AIA) was the first animal model for RA (Conway et al. 1995). Male Lewis rats are injected with Freund's complete adjuvant which is prepared by adding 100 mg *Mycobacterium tuberculosis* to 15.6 ml heavy paraffin oil followed by addition of 1 ml saline. The mixture is then emulsified by pulsing for 5 min with a polytron. Each rat is injected intradermally at the tail base with 300 μg of *Mycobacterium* in a 0.05-ml volume. Paw inflammation is quantified by measuring paw diameter with calipers. The AIA model is useful in preclinical studies to evaluate drug efficacy, particularly for drugs that interfere with T cell function.

Antibody-Induced Arthritis

K/BxN mice express a transgene-encoded T cell receptor (TCR) that is reactive to a self-peptide derived from the ubiquitously expressed enzyme glucose-6-phosphate isomerase (GPI), presented by the MHC class II molecule. K/BxN mice spontaneously develop arthritis with many features similar to RA (Ji et al. 2001). Transfer of serum from arthritic K/BxN mice into healthy non-transgenic animals induces arthritis in a highly reproducible fashion. K/BxN serum pools are prepared from arthritic mice at 60 days of age. Arthritis is induced in diverse recipients by intraperitoneal injection of 7.5 µl serum per g weight diluted in 200 µl total volume at days 0 and 2. A major attraction of this model is to focus on the inflammatory effector phase of disease, without the complicating influences of the autoimmune initiation phase.

Proteoglycan-Induced Arthritis

Proteoglycan (PG)is isolated from cartilage of osteoarthritic patients undergoing joint replacement surgery (Glant et al. 2011). Mice are immunized with PG, 100 µg PG core protein emulsified with 2 mg dimethyl-dioctadecyl ammonium bromide (DDA) adjuvant in phosphate-buffered saline. Mice are immunized intraperitoneally on days 0, 21, and 42 with human cartilage PG emulsified in the synthetic adjuvant DDA. Arthritis severity is determined using a visual scoring system and histological examination. In the mice, such as C57BL/6J and BALB/c, female mice are more susceptible than male mice.

TNF-Induced Arthritis

Intra-articular injection of TNF-alpha induces arthritis (Deng et al. 2005). TNF-induced arthritis is useful for testing the effects of TNF inhibiting biologics. Prior to intra-articular injection, mice and rats are anesthetized with mixture of Hypnorm, Dormicum, and distilled water (1:1:2) and placed on a clean table. After disinfection of the injection area, TNF in 20 µl of PBS is injected intra-articularly into the knee joint. The mice are sacrificed and joints are removed for histopathological examination 3 days after intra-articular injection.

Human TNF-Alpha Transgenic Mice

Transgenic mice overexpressing the human TNF-alpha gene spontaneously develop arthritis (Keffer et al. 1991). A transgenic (Tg) mouse overexpressing human TNF-alpha has been generated. The TNF-Tg mouse develops an erosive polyarthritis with many characteristics of rheumatoid arthritis patients. The TNF-Tg mice are useful tools for dissecting the molecular mechanisms of the pathogenic process and evaluating the efficacy of novel therapeutic strategies for rheumatoid arthritis.

Cross-References

▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Conway JG, Wakefield JA, Brown RH, et al. Inhibition of cartilage and bone destruction in adjuvant arthritis in the rat by a matrix metalloproteinase inhibitor. J Exp Med. 1995;185:449–57.

Deng GM, Zheng LX, Chan FK, Lenardo M. Amelioration of inflammatory arthritis by targeting the pre-ligand assembly domain of tumor necrosis factor receptors. Nat Med. 2005;10:1066–72.

Feldmann M, Maini RN. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. Nat Med. 2003;9:1245–50.

Glant TT, Radacs M, Nagyeri G, et al. Proteoglycan-induced arthritis and recombinant human proteoglycan aggrecan G1 domain-induced arthritis in BALB/c mice resembling two subtypes of rheumatoid arthritis. Arthritis Rheum. 2011;63:1312–21.

Ji H, Gauguier D, Ohmura K, et al. Genetic influences on the end-stage effector phase of arthritis. J Exp Med. 2001;6:321–30.

Keffer J, Probert L, Cazlaris H, et al. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J. 1991;10:4025–31.

Animal Models of Autoimmune Hepatitis

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Synonyms

Animal model; Autoantibodies; Autoimmune hepatitis; Fibrosis; Hepatic tolerance; Intrahepatic T cells; Portal infiltrates

Definition

Human autoimmune hepatitis (AIH) is characterized by specific autoantibodies, elevation of serum aminotransaminases and gamma globulins, liver histopathology suggestive of AIH, and exclusion of other chronic liver diseases. The disease requires lifelong immunosuppression in most cases. Due to limited knowledge about the onset and course of disease, because of the late diagnosis, the limited availability of liver tissue and the required chronic immunosuppression with potentially severe side effects, there is strong need for suitable animal models that reflect AIH.

Introduction

Autoimmune hepatitis (AIH) is a severe and chronic autoimmune inflammation of the liver. The disease is normally diagnosed during advanced stages of disease. Therefore, knowledge about the onset and development of disease is very limited. Therapeutic intervention involves the restricted use of steroids that causes significant side effects. For this reason, there is an urgent to develop a suitable animal model that mirrors the main diagnostic criteria and course of AIH. The diagnostic criteria for AIH can be grouped into clinical, histological, and laboratory evidences.

Disease manifests more often in younger patients with an additional genetic prevalence for females in AIH. In addition to sex and age, some genetic factors predispose to AIH. These include MHC (Czaja et al. 1993), CTLA-4 (Djilali-Saiah et al. 2001), and TNF-α (Cookson et al. 1999) polymorphisms.

Environmental factors have also been suggested to be critical for the initiation of AIH. But due to the time lag between onset and diagnosis, it is difficult to conclude that infections preceding the diagnosis of AIH started liver autoimmunity. In the majority of patients, the induction of AIH has not been linked to a single environmental agent. The course of AIH is chronic and requires therapeutic intervention. 80–90 % of the patients can be treated with a life-long standard treatment regime using steroids, often in combination with azathioprine.

Diagnosis of AIH requires excluding other liver diseases such as PBC, PSC, and viral hepatitis. Histological features suggestive for AIH include lymphoplasmacellular portal infiltrates with interface hepatitis as well as intralobular inflammation.

Diagnosis of AIH is generally confirmed by histopathology and by testing the serum for elevated serum aminotransferases, increased IgG, and autoantibodies that allow differentiation into two clinical subtypes (AIH type I or type II).

An ideal mouse model of AIH should reflect these disease-specific criteria. Unfortunately, most mouse models of AIH display some aspects of liver-specific immunity without developing chronic autoimmune hepatitis.

Acute Liver Damage Induced by Cytokines

Treatment of mice with the ConA lectin leads to polyclonal antigen-independent activation and proliferation of T cells associated with a hepatic

cytokine storm that resulted in severe liver damage with transient elevated aminotransaminases (Tiegs et al. 1992). The inflammatory cytokines IFN-γ and TNF-α have been shown to be critical in ConA-induced liver damage. Neutralizing antibodies against IFN-γ or TNF-α prevented hepatic injury. In this model, $CD8^+$ T cells triggered severe inflammation that led to apoptosis of hepatocytes and fulminant hepatic failure (Tiegs 1997). Intrahepatic $CD4^+$ T cells and macrophages were responsible for the release of T_H1 cytokines (Gantner et al. 1996).

The ConA model explains the role of adaptive and innate immune cells in the liver such as LSEC and NKT cells. LSEC bind ConA preferentially and become targets of $CD4^+$ T cells resulting in their destruction (Knolle et al. 1996). There is strong evidence that FasL-expressing NKT cells are crucial for ConA-induced liver damage. Mice lacking CD1 showed a severe reduction of NKT cells due to the loss of the invariant NKT subpopulation. ConA-mediated hepatic injury was not observed in CD1-deficient mice (Takeda et al. 2000) but could be restored following adoptive transfer of intrahepatic NKT cells.

Thus, the ConA hepatitis model is a model of acute liver injury mediated by a cytokine storm associated with a strong T_H1 response but is not a model of chronic and progressive AIH.

Breaking Liver Tolerance by Immunization

Immunization of mice with liver extracts provided the first evidence that liver tolerance could be broken in mice. Many important and seminal studies with various immunization strategies and antigens were conducted in different mouse strains and have been discussed in detail elsewhere (Jaeckel 2002).

In a series of studies, Mori et al. (Mori et al. 1984) showed that C57BL/6 mice immunized with a syngeneic supernatant of liver homogenate centrifuged at 100 kg were more susceptible to experimental hepatitis than other mouse strains suggesting an important role of the genetic background. Adoptive transfer of LPS-treated activated splenocytes from these recipients to naive recipient mice transferred autoimmunity. This effect was only observed when splenocytes contained T cells, demonstrating the critical role of T cells in the development of AIH.

Lohse et al. used a modified protocol to confirm that the C57BL/6 strain was more susceptible to develop experimental AIH than other strains (Lohse et al. 1990, 1992). In addition, these authors observed a slight increase in serum aminotransferases. The disease could also be adoptively transferred to new recipients by activated splenocytes. But autoantibodies in the serum of recipient animals displayed different specificities than those described in the serum of AIH patients. The group confirmed also the paramount role of T cells in disease histology and pathology and showed that some T cells recognized hepatocyte-expressed antigens. In addition, they demonstrated that splenocytes isolated from mice developing experimental AIH developed some antigen-dependent and independent regulatory properties.

The immunization-induced AIH models brought new insights into the role of the genetic background in disease susceptibility and the critical role of T cells in the onset of hepatitis. Additionally, these studies revealed for the first time the regulatory function of leukocytes in hepatic tolerance and suggested that this regulation is critical to explain the chronic relapsing course of human AIH.

Transgenic Animals in Autoimmune Hepatitis Research

Transgenic models have been used to model AIH. In most models, transgenic mice expressing well-defined neoantigens in the liver were crossed with transgenic mice expressing a T cell receptor specific for the liver-expressed antigen. These models have several advantages over the models described above: (i) liver injury is mediated by T cells rather than cytokines, (ii) the T cell subset responsible for hepatitis is monoclonal rather than polyclonal, and (iii) the

autoantigen is known. However, a key problem in this type of models is that most of liver-specific neoantigens are also expressed in thymus thereby leading to negative selection of antigen-specific transgenic T cells (Klein and Kyewski 2000).

Jones-Youngblood et al. expressed a transgenic MHC I-like molecule Q10 in the liver (Jones-Youngblood et al. 1990). Transgenic mice did not develop spontaneous hepatitis, and liver damage could not even be observed by priming these mice with APCs expressing the Q10/H-2L^{d} fusion protein. Although cross-reactive CD8^{+} T cells recognizing the fusion protein were detected in transgenic mice, their proliferative capacity was reduced compared to CD8^{+} T cells from wild-type mice. T cells isolated from naive, non-transgenic mice caused periportal infiltrates after activation and adoptive transfer into Q10/H-2L^{d}-transgenic recipient mice. Although these results suggest that Q10/H-2L^{d} neoantigen expressing hepatocytes induced tolerance to antigen-specific T cells (Jones-Youngblood et al. 1990), anergy and thymic tolerance could not be excluded.

Similar results were obtained in transgenic mice expressing the allo-MHC molecule H-2K^{b} in hepatocytes (Morahan et al. 1989). Transgenic mice irradiated and reconstituted with non-transgenic bone marrow did not develop hepatitis (Morahan et al. 1989). However, adoptive transfer of non-transgenic lymphocytes into transgenic recipients resulted in severe hepatitis that lasted for up to three months, suggesting that neoantigens could act as alloantigens (Morahan et al. 1989).

Chisari and colleagues obtained similar results in transgenic mice expressing the surface antigen of the hepatitis B virus in hepatocytes (Moriyama et al. 1990). While transgenic mice did not develop hepatitis spontaneously, the transfer of primed non-transgenic T cells into HBV-transgenic mice expressing the neoantigen on hepatocytes caused elevation of serum amino-transferases, antigen-specific autoantibodies, and lymphatic liver infiltrates. Interestingly, the transfer of serum alone induced a mild hepatitis flair. Although this model allows to study the pathogenesis of CD8^{+} T cell-mediated liver damage, it is not a spontaneous model of AIH. Furthermore it remains unclear which mechanisms induce and maintain tolerance to the neoantigen. As circulating neoantigens were detectable in this model, it is not clear whether tolerance was only mediated by hepatocytes (Moriyama et al. 1990; Wirth et al. 1995) or whether tolerance was induced at other sites.

An interesting study used HLA-DRB1*0301 transgenic "humanized" mice immunized with SLA/LP (Mix et al. 2008), which developed SLA/LP-specific autoantibodies. T cells clones isolated from these mice recognized epitopes overlapping with the human autoantibody recognition site of SLA/LP. Although this model did not mimic human AIH, the results allowed the monitoring of autoreactive T cells in AIH patients using newly developed tetramers.

Bertolino and colleagues followed the fate of adoptively transferred H-2K^{b}-specific CD8 transgenic T cells activated intrahepatically in mice expressing the transgenic MHC I molecule H2-K^{b} in hepatocytes. Although hepatitis developed by recipient mice was short lived, they showed that activated CD8^{+} T cells were specifically retained and activated in the liver but underwent Bim-dependent apoptosis (Holz et al. 2008).

In another model TCR transgenic CD8^{+} T cells recognizing LCMVgp33 in the context of H-2D^{b} ignored the transgenic glycoprotein 33 (gp33) epitope of lymphocytic choriomeningitis virus (LCMV) expressed in hepatocytes under the control of the liver-specific albumin (alb) promoter (Voehringer et al. 2000). This ignorance might be due to ineffective cross-presentation of the gp33 antigen. Infection of mice with LCMV was required to break liver tolerance in this model, resulting in a mild and self-limited hepatitis characterized by elevated serum aminotransaminases.

In one of the most recent studies, ovalbumin (OVA) was expressed under the control of the albumin promoter (Buxbaum et al. 2008). These Alb-OVA mice were tolerant to ovalbumin as a result of intrathymic antigen expression and negative selection of anti-OVA T cells. As observed in other models, adoptive transfer of OVA-specific CD4^{+} (OT-II) and CD8+ (OT-I)

T cells into Alb-OVA mice resulted in self-limited liver inflammation. OT-I cells were required for the development of hepatic infiltrates, while OVA-specific $CD4^+$ T cells were dispensable, but enhancers of the $CD8^+$ T cell driven immune response. Other groups had obtained similar results using other liver-specific promoters (Derkow et al. 2007; Zierden et al. 2010).

The importance of LSECs for liver tolerance induction and maintenance was demonstrated in a series of studies by Knolle and coworkers that will be reviewed in the entry "▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance."

In summary, although neoantigen expressing transgenic mouse models pushed our knowledge of liver tolerance and hepatic immunology forward, it is not clear whether they model the response of T cells specific for naturally occurring autoantigens that are expressed to a much lower level than a transgene. In addition, autoreactive T cells have a much lower precursor frequency and a lower affinity to self-antigen than TCR transgenic T cells used in most models.

New Approaches to Develop Preclinical AIH Models

Chronic liver damage is a critical criteria for any AIH animal model. Most transgenic models described above are models of acute hepatitis that never evolved into chronic liver autoimmunity mimicking human AIH.

Most studies focused on T cell-hepatocyte or T cell-LSEC interaction but ignored other critical aspects of AIH such as the impact of genetic background and environmental factors for triggering onset of disease, the role of autoantibodies, regulatory mechanisms, and therapeutic interventions.

So far, only three models achieved a chronic autoimmune hepatitis in mice. Two used transgenically expressed neoantigens (Holdener et al. 2008; Zierden et al. 2010), while the third expressed transiently in the periphery a human alloantigen (Lapierre et al. 2004).

The transgenic neoantigen model described by Zierden et al. mentioned briefly before expressed HA under the control of the liver-specific albumin promoter in mice (Alb-HA). While adoptive transfer of naive HA-specific TCR transgenic $CD8^+$ T cells (Cl4-TCR) into Alb-HA recipients resulted only in transient hepatitis, double-transgenic Alb-HA/Cl4-TCR mice developed chronic hepatitis and hepatic fibrosis (Zierden et al. 2010). Christen and colleagues developed a transgenic model with the human autoantigen CYP2D6 under the control of its own promoter (CYP2D6-tg). While priming of these mice with adenovirus expressing CYP2D6 (Ad-CYP2D6) failed to induce hepatitis, wild-type FVB/N infected i.p. and i.v. with Ad-CYP2D6 develop a chronic form of severe, autoimmune liver damage with subcapsular fibrosis (Holdener et al. 2008). Alvarez and colleagues injected repeatedly intramuscular plasmids coding for CYP2D6-FTCD under a CMV promoter (CMV-CYP2D6-FTCD) and IL-12 in C57BL/6 mice that developed elevated levels of aminotransferases and showed chronic inflammatory infiltrates in the portal tracts, interface hepatitis and necrosis, but no fibrosis (Lapierre et al. 2004).

In addition to chronic liver damage, other clinical, histological, and laboratory criteria are important to reflect human disease. The prevalence of the disease in young individuals and females on one hand and the genetic predisposition for some MHC variants and polymorphisms of other factors on the other hand are two important criteria. While the Alb-HA/Cl4-TCR model results in hepatitis only in male individuals (Zierden et al. 2010), nothing is known about the causality between the genetic background and the Ad-CYP2D6 and the CMV-CYP2D6-FTCD models (Lapierre et al. 2004; Holdener et al. 2008).

The models described here share histologically the lymphoblastic infiltrates. Hepatitis in the Alb-HA/TCR-HA model was described to be fibrotic in the long term, while mice in the Ad-CYP2D6 model developed subcapsular and interlobular fibrosis. The livers of mice with CMV-CYP2D6-FTCD developed necrosis, but no fibrosis. The cellular populations detected in the Alb-HA/TCR-HA model are transgenic

$CD8^+$ T cells. This is in contrast to the polyspecific lymphocytes in the other models. Antigen-specific T cell responses from the polyspecific repertoire were demonstrated in the Ad-FTCD model as well as in the CMV-CYP2D6-FTCD model.

In the clinic, some laboratories use other criteria than pathology to diagnose of AIH. Disease-specific autoantibodies help to rule out other hepatic diseases and allow AIH subclassification. Furthermore, patients with AIH have elevated serum aminotransferases and hypergammaglobuminemia.

The model that used a self-limited adenovirus infection mimicked well the clinical situation (Holdener et al. 2008). Mice have hypergammaglobulinemia, and among the elevated gamma globulins are antigen-specific autoantibodies that recognize the antigen that was used to trigger the disease. None of the models have serum ALT of more than 100U/l after the acute phase during the chronic course of the disease raising the question whether aminotransferases are an adequate readout for chronic mouse models.

AIH mouse models need to be evaluated upon their ability to mirror human disease, to explain mechanisms that are observed in patients and, ideally, to enable preclinical trials that use new therapeutic drugs. The models described in this session fulfill some of these criteria. They will be important to understand the pathophysiology of AIH and might allow the development of AIH-specific therapies.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

Cross-References

- ▶ Autoimmune Hepatitis
- ▶ Autoimmune Hepatitis: Pathogenesis, Association with Other Syndromes
- ▶ CTLA-4
- ▶ Fas/Fas Ligand
- ▶ Innate Immune Cells in the Liver
- ▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance
- ▶ Primary Biliary Cirrhosis, Overview
- ▶ Primary Sclerosing Cholangitis: Clinical and Systemic Manifestations and Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver

References

Buxbaum J, et al. Hepatitis resulting from liver-specific expression and recognition of self-antigen. J Autoimmun. 2008;31(3):208–15.

Cookson S, et al. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. Hepatology. 1999;30(4):851–6.

Czaja AJ, et al. Genetic predispositions for the immunological features of chronic active hepatitis. Hepatology. 1993;18(4):816–22.

Derkow K, et al. Differential priming of CD8 and CD4 T-cells in animal models of autoimmune hepatitis and cholangitis. Hepatology. 2007;46(4):1155–65.

Djilali-Saiah I, et al. CTLA-4/CD 28 region polymorphisms in children from families with autoimmune hepatitis. Hum Immunol. 2001;62(12):1356–62.

Gantner F, et al. T cell stimulus-induced crosstalk between lymphocytes and liver macrophages results in augmented cytokine release. Exp Cell Res. 1996;229(1):137–46.

Holdener M, et al. Breaking tolerance to the natural human liver autoantigen cytochrome P450 2D6 by virus infection. J Exp Med. 2008;205(6):1409–22.

Holz LE, et al. Intrahepatic murine CD8 T-cell activation associates with a distinct phenotype leading to Bim-dependent death. Gastroenterology. 2008;135(3):989–97.

Jaeckel E. Animal models of autoimmune hepatitis. Semin Liver Dis. 2002;22(4):325–38.

Jones-Youngblood SL, et al. Effect of the expression of a hepatocyte-specific MHC molecule in transgenic mice on T cell tolerance. J Immunol. 1990;144(4):1187–95.

Klein L, Kyewski B. "Promiscuous" expression of tissue antigens in the thymus: a key to T-cell tolerance and autoimmunity? J Mol Med. 2000;78(9):483–94.

Knolle PA, et al. Role of sinusoidal endothelial cells of the liver in concanavalin A-induced hepatic injury in mice. Hepatology. 1996;24(4):824–9.

Knolle PA, et al. Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce

differentiation toward Th1 cells. Gastroenterology. 1999;116(6):1428–40.
Lapierre P, et al. A murine model of type 2 autoimmune hepatitis: xenoimmunization with human antigens. Hepatology. 2004;39(4):1066–74.
Limmer A, et al. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med. 2000;6(12):1348–54.
Lohse AW, et al. Experimental autoimmune hepatitis: disease induction, time course and T-cell reactivity. Hepatology. 1990;11(1):24–30.
Lohse AW, et al. Autoantibodies in experimental autoimmune hepatitis. J Hepatol. 1992;14(1):48–53.
Lohse AW, et al. Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells. Gastroenterology. 1996;110(4):1175–81.
Mix H, et al. Identification of CD4 T-cell epitopes in soluble liver antigen/liver pancreas autoantigen in autoimmune hepatitis. Gastroenterology. 2008;135(6):2107–18.
Morahan G, et al. Expression in transgenic mice of class I histocompatibility antigens controlled by the metallothionein promoter. Proc Natl Acad Sci U S A. 1989;86(10):3782–6.
Mori Y, et al. Study of cellular immunity in experimental autoimmune hepatitis in mice. Clin Exp Immunol. 1984;57(1):85–92.
Moriyama T, et al. Immunobiology and pathogenesis of hepatocellular injury in hepatitis B virus transgenic mice. Science. 1990;248(4953):361–4.
Schurich A, et al. Dynamic regulation of CD8 T cell tolerance induction by liver sinusoidal endothelial cells. J Immunol. 2010;184(8):4107–14.
Shimizu Y, et al. Dendritic cell immunization breaks cytotoxic T lymphocyte tolerance in hepatitis B virus transgenic mice. J Immunol. 1998;161(9):4520–9.
Takeda K, et al. Critical contribution of liver natural killer T cells to a murine model of hepatitis. Proc Natl Acad Sci U S A. 2000;97(10):5498–503.
Tiegs G. Experimental hepatitis and role of cytokines. Acta Gastroenterol Belg. 1997;60(2):176–9.
Tiegs G, et al. A T cell-dependent experimental liver injury in mice inducible by concanavalin A. J Clin Invest. 1992;90(1):196–203.
Voehringer D, et al. Break of T cell ignorance to a viral antigen in the liver induces hepatitis. J Immunol. 2000;165(5):2415–22.
Wirth S, et al. Breaking tolerance leads to autoantibody production but not autoimmune liver disease in hepatitis B virus envelope transgenic mice. J Immunol. 1995;154(5):2504–15.
Zierden M, et al. Effects and regulation of autoreactive CD8(+) T Cells in a transgenic mouse model of autoimmune hepatitis. Gastroenterology. 2010;139:975.

Animal Models of Hepatitis B and C

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Synonyms

Animal models; Viral hepatitis

Definition

Infection by the hepatitis B virus (HBV) and hepatitis C virus (HCV) is limited to humans and chimpanzees. Considerable information about the mechanisms that cause viral clearance, viral persistence, and disease pathogenesis has been obtained in the last few decades using chimpanzees infected with HBV and HCV, smaller animal species (woodchuck, duck, Tupaia) infected with related viruses, as well as transgenic or humanized/chimeric mouse models that replicate the viral life cycles to varying degrees.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are prototypic members of the Hepadnaviridae and Flaviviridae families, respectively, which are unique in their capacity to cause persistent infection, cirrhosis, and liver cancer in humans (Guidotti and Chisari 2006). HBV and HCV are considered noncytopathic viruses, and therefore, immune-mediated events play an important role in the pathogenesis and outcome of these infections. The experimental approaches to HBV and HCV pathogenesis have

been difficult because the host range of these viruses is limited to humans and chimpanzees and because of the lack of small animal models that are readily susceptible to infection. Nonetheless, a great deal of information pertaining to the mechanisms that cause viral clearance, viral persistence, and disease pathogenesis has been obtained in the last few decades, especially thanks to the analyses of the natural history and immunobiology of HBV and HCV in chimpanzees and infection studies with related viruses (such as the woodchuck hepatitis virus, WHV, and the duck hepatitis virus, DHBV) in susceptible species. Additional insight has been gained from multidisciplinary studies in transgenic or humanized/chimeric mouse models that replicate the viral life cycles to varying degrees. A concise summary of the contributions that animal models have given to our understanding of HBV and HCV immunobiology and pathogenesis is provided below.

The Chimpanzee Model

Chimpanzees experimentally infected with HBV or HCV develop transient or persistent necroinflammatory liver diseases that are similar to those observed in humans, although usually less severe and seldom evolving toward cirrhotic or cancerous phenotypes (Guidotti and Chisari 2006; Jo et al. 2011). These animals have been instrumental to dissect intrahepatic events that occur early after virus exposure, events that likely determine the outcome of infection (viral clearance versus viral persistence). Owing to these studies, it is clear that both HBV and HCV replicate and spread throughout the liver noncytopathically and that innate immune responses do not significantly contribute to the control of viremia or to the pathogenesis of liver disease (Guidotti and Chisari 2006). Notably, HBV and HCV are sensed very differently by innate defense mechanisms. HBV acts like a stealth virus remaining virtually undetected until the onset of adaptive immune responses, while HCV readily induces interferons and interferon-responsive genes but appears to be resistant to their antiviral effects (Wieland and Chisari 2005).

Perhaps the most important results obtained in chimpanzees infected by HBV or HCV relate to the pathogenic and antiviral roles that adaptive immune responses play during these infections. Indeed, the beginning of liver injury coincides with the entry of virus-specific $CD8^+$ T cells into the liver of chimpanzees infected by HBV or HCV (Guidotti et al. 1999), and depletion of these cells at the peak of viremia delays the onset of biochemical, histological, and clinical evidence of viral hepatitis (Thimme et al. 2003). Although associated with temporary control of viremia, these $CD8^+$ T-cell responses are usually transient in nature suggesting that, when it occurs, the initial priming and expansion of virus-specific $CD8^+$ T cells does not automatically guarantee sustained viral clearance. Rather, permanent virus control appears to require functional $CD4^+$ T-cell responses that are sustained over relatively long periods of time (Shoukry et al. 2004), indicating that virus-specific $CD4^+$ T cells contribute to disease outcome particularly at the level of $CD8^+$ T-cell induction and the establishment or maintenance of a memory cell pool.

Interestingly, humoral immunity is not kinetically associated with the early phases of HBV clearance in chimpanzees, as HBV-specific antibodies (Abs) emerge in these animals several weeks after resolution of a self-limited infection. The protective function of such Ab response is underlined by the notion that chimpanzees that resolved an acute HBV infection are completely protected from rechallenge (Guidotti and Chisari 2006). The observation that complete viral clearance (viral sterilization) following clinical recovery from HBV infection may never occur in humans (Rehermann et al. 1996) suggests that a sustained and protective Ab response prevents the reemergence of HBV in patients that resolved the infection. In contrast to HBV, studies in HCV reinfected chimpanzees have revealed that Abs are quite ineffective at protecting from reinfection, presumably because the viruses that

originally elicited them were counter selected, and such Abs are no longer protective for concurrent quasispecies. Protective immunity during HCV infection does emerge, however, and it is thought to be a T-cell-mediated process, as indicated by studies in chimpanzees that previously resolved an acute HCV infection. Indeed, it was shown in these animals that preexisting HCV-specific memory $CD4^+$ and $CD8^+$ T-cell responses are essential to protection from persistent HCV infection upon rechallenge (Shoukry et al. 2004).

Altogether, the use of chimpanzees experimentally infected with HBV or HCV has demonstrated that the adaptive immune response to these viruses plays a crucial role in viral clearance and liver disease. It is therefore reasonable to assume that viral persistence following these infections requires that immune responses must be either not induced or deficient or, if present, they must be overwhelmed, counteracted, or evaded.

Surrogate Animal Models of Infection with Related Viruses

As mentioned earlier, the availability of animal species that can be experimentally manipulated and represent natural hosts of HBV-related hepadnaviruses has created opportunity for investigation throughout the years. As a general rule these models are not ideal to study immunological phenomena pertaining to viral pathogenesis, mostly because they are outbred species whose immune system is far from being fully characterized. Nevertheless, relevant information has been produced, and it is briefly summarized below.

The Woodchuck model. Woodchucks are rodents that belong to the group of marmots (Marmota) and are susceptible of infection with the woodchuck hepatitis virus (WHV). Like chimps experimentally infected with HBV, woodchucks experimentally infected with WHV develop transient or persistent liver diseases resembling those observed in HBV-infected humans (Menne and Cote 2007). Moreover, persistent liver disease in these animals is tightly linked to the onset of primary liver cancer (hepatocellular carcinoma, HCC) (Menne and Cote 2007). Because of such similarities, WHV-infected woodchucks have been extensively used to uncover the natural history of virologic responses associated with neonatal or adult infection and to study safety and efficacy of vaccines and antiviral therapies (Menne and Cote 2007). We also owe to WHV-infected woodchucks various basic principles by which hepadnaviruses express their genome, replicate within susceptible cells, and promote hepatocellular transformation (Menne and Cote 2007). Notably, immunological tools determining a number of parameters related to T-cell responses in WHV infection have been introduced in recent years. These reagents allowed confirming results obtained in chimps and eased the evaluation of therapeutic vaccines against chronic hepadnaviral infections (Roggendorf et al. 2010).

The Duck model. The fact that DHBV shares fundamental features with HBV has allowed investigators to take advantage of DHBV-based infection systems to extend our comprehension of the molecular biology of HBV and its infectious cycle. Indeed, a variety of the principles of hepadnavirus replication have been established using DHBV, thanks to the experimental infections of ducks with wild-type and mutant viruses in vivo and in cultured primary hepatocytes, and the development of in vitro systems to study biochemically the intricate mechanism of hepadnaviral replication (Schultz et al. 2004). Although DHBV causes both acute and chronic infections in ducks, this virus does not appear to induce severe liver diseases in its host (Jilbert and Kotlarski 2000), thus limiting somehow the relevance of performing studies related to HBV pathogenesis. As per the woodchuck case, the advent of immunological tools detecting immune responses in DHBV-infected ducks has allowed relevant work pertaining to the evaluation of

preventive and therapeutic vaccines against chronic hepadnaviral infection (Jilbert and Kotlarski 2000).

Other models. Tree shrews (Tupaia belangeri) are non-rodent, primate-like, small animals whose hepatocytes are susceptible to infection with HBV and HCV (Cao et al. 2003). The notion that the extent of viral replication in vivo is usually very low coupled with the poor availability of species-specific immune reagents has limited thus far the use of these animals in studies of HBV or HCV pathogenesis. It is also of note that members of the Flaviviridae family such as the bovine viral diarrhea virus (BVDV) or the classical swine fever virus (CSFV) share a similar structural organization with HCV and cause chronic long-term infections in their respective hosts. Although BVDV and CSFV are not generally utilized to infect cattle or pigs experimentally, the manipulation of these viruses in vitro has helped the dissection of various molecular aspects of the HCV life cycle, and it has been used for the evaluation of antiviral agents (Buckwold et al. 2003).

Altogether, the use of surrogate animal models has certainly contributed to clarify and confirm mechanistically several aspects of viral pathogenesis (particularly HBV pathogenesis) and disease outcome. Future utilization of such models for immunological and immunopathological studies will mostly depend on the capacity/will of the scientific community to better characterize their immune system.

Transgenic, Humanized, or Chimeric Mouse Models

Definitive analyses of the immunological mechanisms involved in HBV or HCV pathogenesis required the development of easy-to-manipulate mouse models with a well-defined immune system. Thus far, a large amount of the cellular/molecular pathogenic or antiviral mechanisms that may occur during viral hepatitis in man has been obtained in transgenic mice replicating HBV at high levels in their liver (Guidotti et al. 1995). Up until recently, the absence of immunocompetent mice replicating HCV has hindered similar analyses in the HCV field. However, the very recent establishment of an immunocompetent humanized mouse model capable of sustaining HCV infection and replication in mouse hepatocytes (Dorner et al. 2011) has the potential to rapidly change this scenario, allowing to confirm/dispute whether pathogenic and antiviral mechanisms relevant to HBV (see below) are also operative during HCV infection. Additional pathogenic insight has emerged from the study of mice whose liver expresses structural and nonstructural HBV or HCV genes individually and from chimeric mice in which human hepatocytes repopulate the liver and support HBV or HCV infection/replication. Results that emerged from the models abovementioned are summarized below.

HBV and HCV transgenic mice. Possibly, the most relevant results obtained in HBV-replication-competent transgenic mice relate to the dissection of highly complex but coordinated processes that regulate the host immune response to this virus. Starting with the fundamental role that effector virus-specific $CD8^+$ T cells play in the pathogenesis of viral clearance and liver disease, it was shown that the adoptive transfer of these cells into HBV-replication-competent transgenic mice triggers a necroinflammatory liver disease which results in the inhibition of HBV replication and shares the same histologic features of acute viral hepatitis in man (Guidotti and Chisari 2006). These studies in mice have also taught us that the antiviral potential of virus-specific $CD8^+$ T cells is largely mediated by noncytolytic mechanisms involving the local production of IFN-gamma by these cells. Indeed, it has been reported that IFN-gamma (mostly via its capacity to induce nitric oxide in the liver) prevents the assembly of replication-competent HBV RNA-containing capsids in the hepatocyte in a proteasome- and kinase-dependent manner (Guidotti and Chisari 2006). During this process, the viral nucleocapsids

disappear from the cytoplasm of the hepatocytes, and the viral RNAs are destabilized by a SSB/La-dependent mechanism in the nucleus, yet the hepatocytes remain perfectly healthy. The notion that IFN-gamma produced by activated $CD8^+$ T cells plays a direct role in viral clearance is corroborated by studies in chimpanzees acutely infected with HBV (Guidotti and Chisari 2006).

As virus-specific $CD8^+$ T cells reach the liver parenchyma, the first step in the disease process is antigen recognition by these cells, which rapidly induces hepatocellular apoptosis. The initial apoptotic process, however, involves a relatively small number of hepatocytes. As time progresses, many antigen nonspecific polymorphonuclear and mononuclear inflammatory cells are recruited into the liver, and they contribute to worsen disease severity (Kakimi et al. 2001). While the recruitment of antigen nonspecific mononuclear cells is mostly a chemokine-dependent event, the recruitment of antigen nonspecific polymorphonuclear cells largely relies on the recognition of hepatocellular damage. Studies have indeed shown that high-mobility group box 1 (HMGB1) protein, an abundant nuclear protein acting as an architectural chromatin-binding factor, can be passively released by necrotic (but not apoptotic) hepatocytes and chemoattract polymorphonuclear cells (Sitia et al. 2011). Notably, the recruitment process of virus-specific $CD8^+$ T cells into the liver is quite different from that of antigen nonspecific inflammatory cells, and it largely depends on platelets capable of becoming activated within the liver microvasculature (Iannacone et al. 2005).

Studies in HBV transgenic mice also helped us in characterizing the immune-mediated basis of hepatocellular transformation during chronic hepatitis. It was shown that the maintenance of low-level liver cell destruction caused by a dysfunctional and detrimental virus-specific $CD8^+$ T-cell response (unable to completely clear HBV antigens from the liver) is sufficient to cause HCC development (Nakamoto et al. 1998). According to these results, chronic viral hepatitis could be viewed as T-cell-dependent premalignant state promoting processes, like hepatocellular regeneration (i.e., cellular DNA synthesis) and inflammation (i.e., production of mutagens) that are oncogenic and result in the random and multiple genetic/chromosomal alterations responsible for HCC development.

Finally, it is worth mentioning that additional information related to how specific HBV or HCV gene products may influence the pathogenesis of these infections has been developed in transgenic mice that hepatocellularly express structural and nonstructural HBV or HCV genes individually. A variety of phenotypes have been reported, and they include mice that spontaneously develop chronic hepatitis, fibrosis, steatosis, cirrhosis, or HCC (Guidotti and Chisari 2006). It must be noted, however, that most of the studies have been obtained in animals that express viral products at levels that are much higher than those detected during natural infection in humans and, therefore, further work needs to be performed in order to understand the relevance of these observations.

Humanized and chimeric mice. As stated above, the absence of immunocompetent mice replicating HCV has held back studies on HCV immunobiology and pathogenesis for a number of years. Interestingly, however, it has been recently shown that the in vivo hepatocellular expression of the HCV coreceptors CD81 and occludin renders fully immunocompetent inbred mice permissive to HCV infection (Dorner et al. 2011). It is expected that this important breakthrough will open important opportunities for studying HCV pathogenesis and immunobiology in the future. As also stated above, different investigators have used chimeric mice in which human hepatocytes repopulate the liver and support HBV or HCV infection/replication (Meuleman and Leroux-Roels 2008). Besides limitations that include high costs, low throughput, and significant individual variability in viral replication levels, the fact that these chimeric mice must be maintained under strict immunocompromised conditions renders them not particularly suited for immunological and immunopathological studies. Whether this model will significantly improve thanks to the simultaneous implantation of a fully functional immune system of human origin remains to be determined.

Concluding Remarks

Our comprehension of the pathogenesis of HBV and HCV infection has significantly advanced in recent years, particularly because of the experimental use of animal models. In spite of these accomplishments, however, many mechanistic issues pertaining to the immunopathogenesis of chronic HBV or HCV infection remain unresolved, especially those that are linked to the development of cirrhosis, steatosis, or cancer. Addressing such issues in animal models will be challenging but certainly important for the design of novel approaches to treat these life-threatening diseases.

Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Adaptive Immune Cells in the Liver
- ▶ Cytotoxic T Lymphocytes
- ▶ Immune Responses to the Hepatitis C Virus
- ▶ Innate Immune Cells in the Liver
- ▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance
- ▶ Platelets, Atherosclerosis, and Immunity
- ▶ Primary T-Cell Activation in Liver

References

Buckwold VE, Beer BE, Donis RO. Bovine viral diarrhea virus as a surrogate model of hepatitis C virus for the evaluation of antiviral agents. Antiviral Res. 2003;60:1–15.

Cao J, Yang EB, Su JJ, Li Y, Chow P. The tree shrews: adjuncts and alternatives to primates as models for biomedical research. J Med Primatol. 2003;32:123–30.

Dorner M, Horwitz JA, Robbins JB, Barry WT, Feng Q, Mu K, Jones CT, Schoggins JW, Catanese MT, Burton DR, Law M, Rice CM, Ploss A. A genetically humanized mouse model for hepatitis C virus infection. Nature. 2011;474:208–11.

Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. Annu Rev Pathol. 2006;1:23–61.

Guidotti LG, Matzke B, Schaller H, Chisari FV. High-level hepatitis B virus replication in transgenic mice. J Virol. 1995;69:6158–69.

Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. Science. 1999;284:825–9.

Iannacone M, Sitia G, Isogawa M, Marchese P, Castro MG, Lowenstein PR, Chisari FV, Ruggeri ZM, Guidotti LG. Platelets mediate cytotoxic T lymphocyte-induced liver damage. Nat Med. 2005;11:1167–9.

Jilbert AR, Kotlarski I. Immune responses to duck hepatitis B virus infection. Dev Comp Immunol. 2000;24:285–302.

Jo J, Lohmann V, Bartenschlager R, Thimme R. Experimental models to study the immunobiology of hepatitis C virus. J Gen Virol. 2011;92:477–93.

Kakimi K, Lane TE, Wieland S, Asensio VC, Campbell IL, Chisari FV, Guidotti LG. Blocking chemokine responsive to gamma-2/interferon (IFN)-gamma inducible protein and monokine induced by IFN-gamma activity in vivo reduces the pathogenetic but not the antiviral potential of hepatitis B virus-specific cytotoxic T lymphocytes. J Exp Med. 2001;194:1755–66.

Menne S, Cote PJ. The woodchuck as an animal model for pathogenesis and therapy of chronic hepatitis B virus infection. World J Gastroenterol. 2007;13:104–24.

Meuleman P, Leroux-Roels G. The human liver-uPA-SCID mouse: a model for the evaluation of antiviral compounds against HBV and HCV. Antiviral Res. 2008;80:231–8.

Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. J Exp Med. 1998;188:341–50.

Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med. 1996;2:1104–8.

Roggendorf M, Yang D, Lu M. The woodchuck: a model for therapeutic vaccination against hepadnaviral infection. Pathol Biol (Paris). 2010;58:308–14.

Schultz U, Grgacic E, Nassal M. Duck hepatitis B virus: an invaluable model system for HBV infection. Adv Virus Res. 2004;63:1–70.

Shoukry NH, Cawthon AG, Walker CM. Cell-mediated immunity and the outcome of hepatitis C virus infection. Annu Rev Microbiol. 2004;58:391–424.

Sitia G, Iannacone M, Aiolfi R, Isogawa M, van Rooijen N, Scozzesi C, Bianchi ME, von Andrian UH, Chisari FV, Guidotti LG. Kupffer cells hasten resolution of liver immunopathology in mouse models of viral hepatitis. PLoS Pathog. 2011;7:e1002061.

Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, Chisari FV. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol. 2003;77:68–76.

Wieland SF, Chisari FV. Stealth and cunning: hepatitis B and hepatitis C viruses. J Virol. 2005;79:9369–80.

Anti-glomerular Basement Membrane Disease

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Synonyms

Goodpasture's disease

Definition

Anti-glomerular basement membrane (anti-GBM) disease, also known as Goodpasture's disease, is a rare but life-threatening condition that is characterized by circulating and deposited antibody directed against the GBM, which presents with features of crescentic glomerulonephritis, with or without concomitant pulmonary hemorrhage.

History

The eponymous term "Goodpasture's Syndrome" was first used by Australians Stanton and Tange (Stanton and Tange 1958) in their 1958 article describing 9 cases of glomerulonephritis in association with pulmonary hemorrhage, in which they acknowledge the American pathologist Ernest Goodpasture with the first description of the syndrome in his 1919 paper on the etiology of influenza (Goodpasture 1919). However, it is not known whether any of these cases had anti-GBM antibodies, as it was not until the development of immunofluorescence techniques in the 1960s that Sheer and Grossman first described the typical linear staining pattern for immunoglobulins in renal tissue seen in this disease. In 1967, Lerner and colleagues showed that these antibodies, eluted from the kidneys of patients, were reactive with normal kidney tissue, and subsequently demonstrated their pathogenic potential by administration to nonhuman primates (Lerner et al. 1967). The first comprehensive clinical report of "anti-GBM disease" was by Wilson and Dixon in 1973 (Wilson and Dixon 1973).

The term "Goodpasture's disease" is now generally reserved for those patients with detectable anti-GBM antibodies in association with renal or pulmonary disease.

Epidemiology and Associations

Goodpasture's disease is a rare disorder, with an approximate incidence of 1 per million population per year. There is a slight male preponderance and bimodal age distribution, with peak incidence in the third and sixth decades. The disease is more common in Caucasian populations, is well represented in Asians, and thought to be rare in those of African origin. Some series have suggested a higher disease incidence in spring and early summer.

Reported environmental associations include cigarette smoking (Donaghy and Rees 1983) and exposure to hydrocarbons and industrial solvents. Associations with other diseases include membranous nephropathy, ANCA-associated vasculitis, and renal stone disease treated with lithotripsy.

In common with other autoimmune diseases, genetic predisposition is believed to be an important determinant of disease development (Zhou et al. 2010), and there is a strong association with human leucocyte antigen (HLA) genes in particular, with approximately 80 % of patients inheriting an HLA-DR2 haplotype. Genotyping studies have revealed a hierarchy of associations with particular DRB1 alleles: DRB1*1501, DRB1*03, and DRB1*04 are positivity associated with disease, whereas there is a negative association with DRB1*01 and DRB1*07 (Fisher et al. 1997). The molecular basis of these HLA associations is not defined, although the observation that the DRB1*01 and DRB1*07 alleles appear to confer a dominant negative effect and that these protective alleles bind peptides from the Goodpasture autoantigen with greater affinity than the positively associated alleles suggests that they might compete for peptide epitopes with the susceptibility alleles.

A link to major histocompatibility complex (MHC) Class II genes has also been reported in murine strains susceptible to experimental disease, although non-MHC genes are also involved since both resistant and susceptible rat strains have been shown to share the same MHC type. In addition, the susceptibility alleles in humans are common in the general population, yet the disease remains exceedingly rare. These observations demonstrate the importance of other, as yet undefined, genetic and environmental factors in the development of disease.

Pathogenesis

Lerner's classic transfer experiments (since repeated in a number of different species) were the first demonstration that autoantibodies could directly cause disease, and the role of humoral immunity and the nature of the target autoantigen have been the predominant focus of historical research interest in this condition. More recent observations, and in particular studies of animal models that manifest a similar pathology, also confirm an important role for cellular immunity, both as orchestrator of the autoimmune response and direct effector of tissue injury.

The Goodpasture Autoantigen

The Goodpasture autoantigen (Pedchenko et al. 2010; Saus et al. 1988; Turner et al. 1992) was first identified as a 27 kDa protein from collagenase-digested GBM preparations, and the key autoantibody target was subsequently defined as the non-collagenous (NC1) domain of the alpha-3 chain of type IV collagen (α3(IV)NC1) found in the basement membrane of the glomerulus and lung (and also retina, choroid plexus, and cochlea). Immunization with either collagenase-solubilized or recombinant forms of the protein from various species induces disease in a number of animal models, confirming the universal antigenicity of this protein.

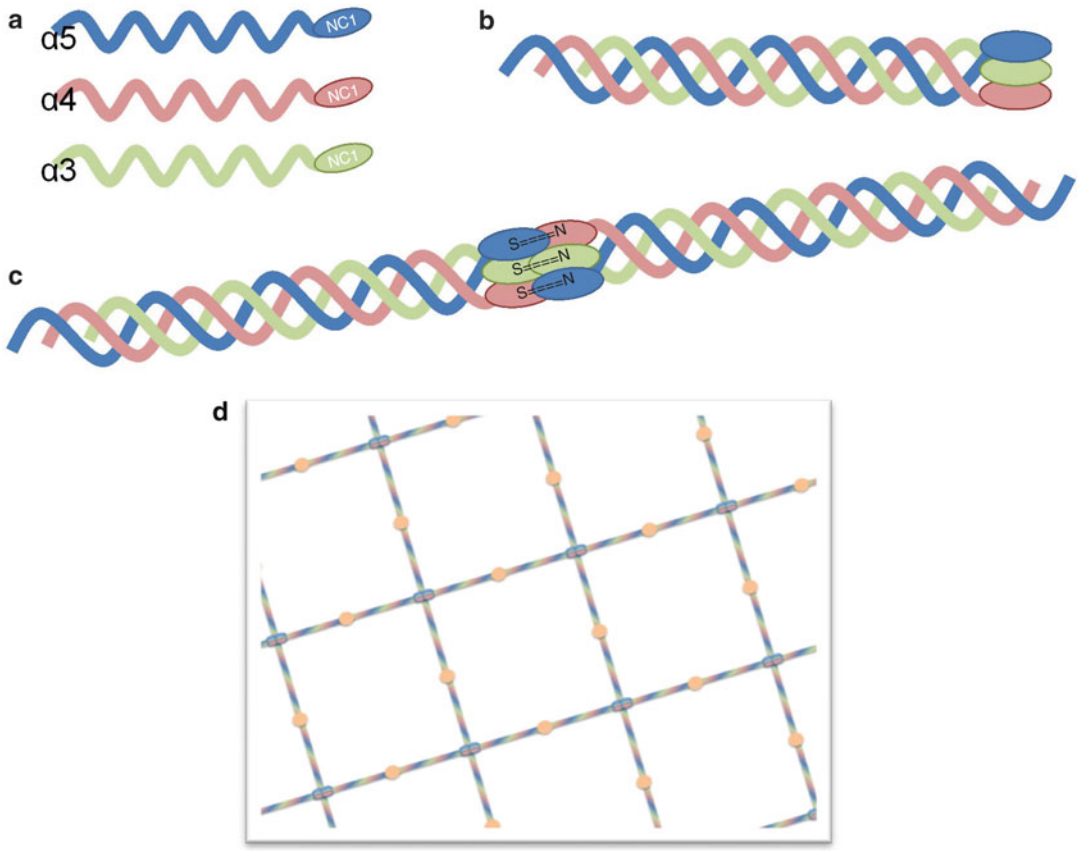
In its native form, the collagen IV network in the GBM consists of triple-helical protomers of α3, α4, and α5 chains. The carboxy-terminal domains of these α3α4α5 protomers form a trimeric "cap," end-to-end association of which results in the formation of the hexameric NC1 domain (Fig. 1). The quaternary structure of this hexamer is stabilized by hydrophobic and hydrophilic interactions across the planar surfaces of opposing trimers and reinforced by sulfilimine bonds cross-linking opposing NC1 domains. Two key autoantibody epitopes within α3(IV)NC1 have been described, designated E_A (incorporating residues 17–31 towards the amino-terminus) and E_B (residues 127–141 towards the carboxy-terminus), which in the native form are sequestered at the junction with α4 and α5 chains within the triple-helical structure.

Humoral Immunity

Sera from all patients with anti-GBM disease recognize α3(IV)NC1, although sera from a proportion of patients may also recognize other collagen chains. Approximately 70 %, for example, will have both circulating and deposited antibodies to the α5(IV)NC1 domain, suggested to arise through a process of "epitope spreading" following primary disease initiation by autoantibodies to α3(IV)NC1. Circulating and deposited antibodies to α3(IC)NC1 recognize both epitopes E_A and E_B, with one study suggesting that antibodies to E_A are more strongly associated with disease outcome.

In addition to the passive transfer models of Lerner and others, several clinical observations support a directly pathogenic role for these autoantibodies in anti-GBM disease. Antibody titer, immunoglobulin subclass, and in at least one study, antibody avidity have been correlated with disease outcome in patient cohorts. In addition, the rapid removal of circulating antibodies by plasmapheresis is associated with better clinical outcomes, and disease recurs rapidly in renal allografts if circulating antibodies are present at the time of transplantation.

The function of other components of the humoral immune system, such as the Fc receptor



Anti-glomerular Basement Membrane Disease, Fig. 1 (**a**) Individual α3, α4, and α5 chains of type IV collagen with carboxy terminal non-collagenous (NC1) domains (**b**) Association of α3, α4, and α5 chains to form triple-helical trimer, with NC1 domain "cap" (**c**) Type IV collagen molecule, showing end-to-end association of NC1 trimers to form NC1 hexamers, with sulfilimine cross-links (S = N), and resultant sequestration of α3 chain epitopes (**d**) Binding through 7s domains (shown in orange) completes the lattice-like structure of the type IV collagen network

and the complement cascade, has also been implicated in the pathogenesis of this disease. Copy number variation of activatory FcγIIIA genes is found at higher frequency in patients compared with healthy controls, and certain FcγIIB receptor polymorphisms are also present at higher frequency in diseased patients. Similarly, copy number variation of the orthologous FcγRIII genes is a determinant of disease susceptibility in the rat (Aitman et al. 2006). Mice deficient in activatory FcγR are protected from a variety of autoimmune diseases, including glomerulonephritis, and FcγIIB (an inhibitory FcR)-deficient mice are also more susceptible to experimental disease. Classical activation of the complement pathway by immune complexes formed by antigen and antibody has also been shown to contribute to murine models of disease, and mice deficient in C3 and C4 components are resistant to nephrotoxic nephritis.

The presence of low-titer "natural" autoantibodies to GBM has been reported in normal populations (Cui et al. 2010). These antibodies recognize the same epitopes as antibodies from patients, although their presence does not result in disease. This may be due to differences in the titer or predominant subclasses of these natural autoantibodies (IgG2 and IgG4 versus IgG1 and IgG3 in disease) or the role of other regulatory factors. The presence of circulating antibody has

been reported to predate the onset of clinical disease by several years (Olson et al. 2011) (although antibody subclass and epitope specificity was not reported in this study), an observation that suggests the involvement of other factors in the development of disease.

Cellular Immunity

Several clinical observations support a role for cell-mediated immunity. CD4+ and CD8+ T cells can be demonstrated in diseased glomeruli in humans, and the presence of class-switched, high-affinity autoantibody, along with a strong HLA-association, implies a requisite for T cell-mediated help. Mononuclear cells from patients have also been shown to proliferate in response to α3(IV)NC1 (as do cells from healthy individuals, though at much lower frequency), and the frequency of autoreactive CD4+ T cells has been shown to correlate with disease activity. The pathogenic T cell epitopes in humans, however, have not been consistently defined.

Studies in various experimental animal models, particularly in rodents, also implicate cellular immune responses in disease pathogenesis. Resistant mouse strains, for example, may develop circulating antibody following immunization with the autoantigen, yet fail to develop glomerulonephritis, suggesting the need for other factors for the full expression of disease. In certain susceptible mouse strains, passive transfer of autoantibody does not result in disease in T cell receptor deficient animals. Likewise, CD4 and CD8 deficient mice are protected from experimental disease, and in rat models a variety of anti-T cell approaches have ameliorated disease – such as the use of cyclosporine A, monoclonal antibodies against CD4 and CD8, and co-stimulation blockade targeted at CD40-CD40L or B7-CD28 interactions (Reynolds et al. 2000).

Studies in animal models also suggest that cell-mediated immunity may have a significant role as a direct effector of tissue injury, in addition to directing the humoral response. In early avian models, mononuclear cells could transfer disease to bursectomized birds. More recently, B cell (μ-chain) deficient mice have been shown to develop glomerular injury following immunization with α3(IV)NC1 (Dean et al. 2005), and experimental disease in rats has been transferred by CD4+ T cells from nephritic rats expanded in vitro by stimulation with α3(IV)NC1 (Wu et al. 2002). In these experiments, animals developed glomerulonephritis in the absence of detectable antibody, suggesting a directly injurious response by cellular immune effectors. However, there is no direct evidence yet of a similar process in human disease.

Tolerance and Autoimmunity to α3(IV)NC1

α3(IV)NC1 is expressed in human thymus, although the finding of natural autoantibodies and T cells reactive to α3(IV)NC1 in normal individuals suggests some failure to achieve central tolerance to this antigen during immunological development. It has been suggested that certain autoreactive T cell peptides are sensitive to rapid enzymatic degradation during antigen processing, limiting the exposure of autoreactive cells to their corresponding antigens and thus allowing them to escape negative selection (Zou et al. 2007). The additional factors that result in further breakdown of tolerance and development of clinical disease are not clear, though may include the need to expose sequestered epitopes within the Goodpasture antigen.

Notably, the cross-linked native hexameric NC1 domain does not bind antibody, and it is thought that disruption of its quaternary structure, with rupture of the sulfilimine cross-links and dissociation of the hexamer, is required to expose the pathogenic epitopes required for autoantibody binding. Both cross-linked and non-cross-linked forms of the hexamer exist in humans and other primates, but only the cross-linked form in mice, perhaps explaining the resistance of some mice strains to passive transfer models of disease. This requirement for "conformational transition" of the autoantigen may also explain the association of anti-GBM disease with other processes

that may damage the basement membrane in the kidney (such as membranous nephropathy, ANCA-associated disease, or lithotripsy) or the lung (such as smoking and inhalation of hydrocarbons), resulting in exposure of usually sequestered epitopes.

The recovery phase of this condition is associated with a progressive fall in autoantibody titer (even in the absence of immunosuppressant treatment) and a lower frequency of CD4+ T cells reactive to α3(IV)NC1, along with development of a regulatory CD25+ T cell subset that may suppress responses to α3(IV)NC1 (Salama et al. 2003). This suggests the re-emergence of immunological tolerance, which may be reflected by the rarity of clinical relapses in this condition.

Clinical Considerations

The great majority of patients will present with features of rapidly progressive glomerulonephritis, which is characterized by an abrupt decline in renal function with an active urinary sediment containing protein and red cell casts. There are a small number of reported cases, however, with only mild renal impairment. Approximately 50 % of patients will have coexistent pulmonary hemorrhage, which may present with hemoptysis and be seen as alveolar shadowing on chest radiography or detected by the finding of an increased carbon monoxide transfer factor. A small number of patients may present with isolated lung disease in the absence of overt renal pathology.

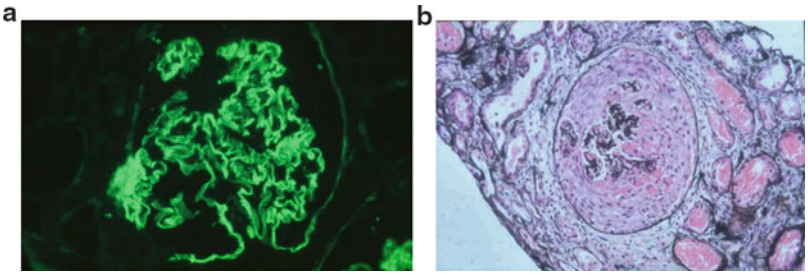
Strictly, the diagnosis of anti-GBM disease rests on the demonstration of:

(a) Circulating anti-GBM antibodies – these are detectable in the majority of patients by conventional techniques such as enzyme-linked immunosorbent assay (ELISA) and Western blotting using purified human or animal GBM preparations. In the small proportion of patients who do not have detectable antibodies using these methods, more sensitive techniques such as biosensor assay using recombinant antigen have been reported to detect circulating antibody.

(b) Deposited anti-GBM antibodies – seen as linear deposits of IgG along the GBM by immunofluorescence techniques (Fig. 2a). The majority of patients will also demonstrate linear staining for complement C3 and some for other antibody isotypes, such as IgA and IgM.

(c) Crescentic glomerulonephritis – seen on standard light microscopy of renal tissue (Fig. 2b). Fibrinoid necrosis may be present. The finding of monophasic injury, where all glomeruli show lesions of similar acuity, is typical.

Standard treatment for anti-GBM disease, first introduced in the 1970s (Lockwood et al. 1976), consists of plasmapheresis, to rapidly remove the pathogenic antibody (and possibly other proinflammatory mediators) from the circulation, and immunosuppression with cyclophosphamide and corticosteroids, which inhibit the production of further autoantibody and reduce end-organ tissue inflammation and damage. Retrospective series suggest this combination of treatment is effective in most patients with serum creatinine <500 micromol/l at presentation, and one large study showed it can be successful even in patients who present with severe renal injury (creatinine >500 micromol/l) but not yet on dialysis (Levy et al. 2001). In the same study, lung hemorrhage responded to treatment in 90 % of patients. One small trial suggested better recovery of renal function when plasmapheresis was used in addition to drug therapy (Johnson et al. 1985); however, given its rarity (and the efficacy of accepted treatments), there are no large randomized controlled trials in this disease. The use of alternative immunosuppressant agents (such as cyclosporine, mycophenolate mofetil, or rituximab) has been reported in small series and individual cases, although there is insufficient evidence to support their use as first-line treatment at present. Poor prognostic features include dialysis dependency at diagnosis and 100 % crescents on renal biopsy, which suggest the patient will not recover independent renal function.



Anti-glomerular Basement Membrane Disease, Fig. 2 (**a**) Renal biopsy immunofluorescence for IgG revealing linear deposits along the glomerular basement membrane and weaker staining of Bowman's capsule (**b**) Renal biopsy light micrograph showing crescent formation, with inflammatory and epithelial cells in Bowman's space compressing the glomerular tuft

Alport Syndrome and Transplantation

The Alport syndrome of progressive renal disease and deafness has been attributed to mutations in any of the three genes which encode the α3, α4, or α5 chains, resulting in a failure to produce the normal type IV collagen network present in the GBM. The most common mutation is in the COL4A5 gene located on the X chromosome, giving the typical X-linked disease. These patients may develop end-stage renal failure requiring renal transplantation. Of these, approximately 5–10 % will develop anti-GBM antibodies post-transplant, due to the development of an alloimmune response to "normal" α3 or α5 chains within the renal graft, seen as a neo-antigen to the recipient immune system. These antibodies do not recognize the individual E_A and E_B epitopes recognized by sera from Goodpasture patients, but rather a composite epitope that is not sequestered within the native hexamer. The development of Goodpasture's disease in this context often results in loss of the renal allograft, and repeated transplantation will invariably lead to disease recurrence and graft loss.

Cross-References

- ▶ Indications for Biopsy in Autoimmune GN
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Vasculitis and the Kidney

References

Aitman TJ, et al. Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. Nature. 2006;439(7078):851–5.

Cui Z, et al. Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. Kidney Int. 2010;78(6):590–7.

Dean EG, et al. Experimental autoimmune Goodpasture's disease: a pathogenetic role for both effector cells and antibody in injury. Kidney Int. 2005;67(2):566–75.

Donaghy M, Rees AJ. Cigarette smoking and lung haemorrhage in glomerulonephritis caused by autoantibodies to glomerular basement membrane. Lancet. 1983;2(8364):1390–3.

Fisher M, et al. Susceptibility to anti-glomerular basement membrane disease is strongly associated with HLA-DRB1 genes. Kidney Int. 1997;51(1):222–9.

Goodpasture EW. The significance of certain pulmonary lesions in relation to the etiology of influenza. Am J Med Sci. 1919;158:863–70.

Johnson JP, et al. Therapy of anti-glomerular basement membrane antibody disease: analysis of prognostic significance of clinical, pathologic and treatment factors. Medicine (Baltimore). 1985;64(4):219–27.

Lerner RA, Glassock RJ, Dixon FJ. The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. J Exp Med. 1967;126(6):989–1004.

Levy JB, et al. Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. Ann Intern Med. 2001;134(11):1033–42.

Lockwood CM, et al. Immunosuppression and plasma-exchange in the treatment of Goodpasture's syndrome. Lancet. 1976;1(7962):711–5.

Olson SW, et al. Asymptomatic autoantibodies associate with future anti-glomerular basement membrane disease. J Am Soc Nephrol. 2011;22(10):1946–52.

Pedchenko V, et al. Molecular architecture of the Goodpasture autoantigen in anti-GBM nephritis. N Engl J Med. 2010;363(4):343–54.
Reynolds J, et al. CD28-B7 blockade prevents the development of experimental autoimmune glomerulonephritis. J Clin Invest. 2000;105(5):643–51.
Salama AD, et al. Regulation by CD25+ lymphocytes of autoantigen-specific T-cell responses in Goodpasture's (anti-GBM) disease. Kidney Int. 2003;64(5):1685–94.
Saus J, et al. Identification of the Goodpasture antigen as the alpha 3(IV) chain of collagen IV. J Biol Chem. 1988;263(26):13374–80.
Stanton MC, Tange JD. Goodpasture's syndrome (pulmonary haemorrhage associated with glomerulonephritis). Australas Ann Med. 1958;7(2):132–44.
Turner N, et al. Molecular cloning of the human Goodpasture antigen demonstrates it to be the alpha 3 chain of type IV collagen. J Clin Invest. 1992;89(2):592–601.
Wilson CB, Dixon FJ. Anti-glomerular basement membrane antibody-induced glomerulonephritis. Kidney Int. 1973;3(2):74–89.
Wu J, et al. CD4(+) T cells specific to a glomerular basement membrane antigen mediate glomerulonephritis. J Clin Invest. 2002;109(4):517–24.
Zhou XJ, et al. Advances in the genetics of anti-glomerular basement membrane disease. Am J Nephrol. 2010;32(5):482–90.
Zou J, et al. Presentation of the Goodpasture autoantigen requires proteolytic unlocking steps that destroy prominent T cell epitopes. J Am Soc Nephrol. 2007;18(3):771–9.

Antioxidants

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Synonyms

Electron donors; Molecules capable of blocking or reversing the oxidation of other molecules; Reducing agents; Thiols, such as glutathione and cysteine

Definition

Antioxidants are molecules that inhibit the formation of oxidative products and prevent or reverse the actions of oxidants. Antioxidants can be exogenous or endogenous small molecules such as N-acetylcysteine and glutathione, respectively, that can neutralize the presence or block the production of oxidants; enzymes that modify the levels of oxidants such as superoxide dismutase, glutathione peroxidase, and catalase; and enzymes that synthesize or regenerate reducing agents, such as γ-glutamylcysteine ligase and glutathione synthase and glutathione reductase, respectively.

Introduction of Reduction-Oxidation (Redox) and Immunity

Although lymphocytes have been known to be more sensitive to oxidative stress than many other cell types, the subcellular mechanisms responsible for their sensitivity and for the differential oxidative sensitivities among the lymphoid and myeloid subsets have not been fully elucidated. There is an extensive amount of literature that documents a decline in the immune capacity of older individuals (>65 years), especially with regard to a loss or dysregulation of T cell functions (Murasko and Goonewardene 1990; Ben-Yehuda and Weksler 1992). Likewise, there have been numerous theories and reports that link aging with oxidative stress (Sohal and Orr 1992; Harman 2006). Oxidative stress is a consequence of an excess of oxidants, such as reactive oxygen species (ROS), and/or a deficit of antioxidants. However, oxidative stress does not equally affect all antioxidants at equivalent rates, e.g., the most abundant antioxidants in plasma are cysteine and glutathione (GSH), and both are converted to their oxidized partners (cystine and glutathione disulfide, GSSG) by the increase in oxidative stress that occurs with age, but the loss of cysteine and GSH occurs at different rates. Oxidative stress can modify DNA/RNA, protein, carbohydrate, and lipid structure and function, and reduction-oxidation (redox) processes play important regulatory roles in cellular signaling, proliferation, and apoptosis. Aging and the concomitant immunosenescence are likely due to genetic and environmental causes. However,

oxidative stress and inflammatory processes, which are closely connected, are associated with disorders that accompany aging, including immunopathologies. Thus, the prevalence of immunopathologies, such as rheumatoid arthritis, multiple sclerosis, and leukemias, is a posited consequence of age-related redox changes.

Depletion of cellular thiols can result in immunomodulation due to a restructuring of plasma membrane (PM) lipids and proteins (surface receptors and their signal transduction circuits) of lymphocytes and antigen-presenting cells (APC), which may alter early signal transduction events (Go to G1) associated with APC-T cell interactions and subsequent events, including proliferation and the production of different types of cytokines. Depletion of cellular thiols (changes of the thiol-reactive proteins and nonproteins, such as cysteine and GSH) results in immunomodulation with lowered antigen-specific lymphocyte responses, but with an elevated level of some innate immune responses causing inflammation with elevations of inflammatory cytokines (interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)α), as a result of signaling changes that occur with age. Cellular thiol proteins can influence membrane lipid domains (Bergman et al. 1984), membrane lipids can influence the number of cellular thiols (Schacter et al. 1983), and oxidative stress can directly alter the lipid composition of the PM via lipid peroxidation (Hu and Tappel 1992; McIntyre and Hazen 2010). All of these actions are directly or indirectly related to inflammatory processes. Endogenous antioxidant melatonin and dietary antioxidants, including vitamin E (tocopherols) and lipids, such as eicosapentaenoic acid, docosahexaenoic acid, and γ-linolenic acid have modulatory effects on the generation of lipid peroxides due, in part, to their lipophilic nature. The differential compartmentalization of endogeneous and dietary antioxidants, cellular thiols, and the enzymes (Go and Jones 2008), including thioredoxins (Trx1 and Trx2), glutaredoxin, and peroxiredoxins, that influence ROS and protein and nonprotein thiols and the dependent and independent relationships between these redox modifiers (molecules that regulate reduction-oxidation reactions) are the complexities that delay understanding of antioxidant influences on immunity. However, it is clear that redox pathways (thiol/disulfide exchange reactions) play a key role in immune cell activities and survival (Perl et al. 2002), e.g., signaling molecules apoptosis signal-regulating factor-1 (Ask-1), nuclear factor-2 (Nrf2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) are regulated differentially by Trx1 and GSH (Go and Jones 2008).

Thiol Levels Differentially Affect T Cell Subset Activation

The topographical distribution of surface molecules, such as the T cell antigen-specific receptor (TCR), the CD3 complex, and CD4 and their association with the lipid domains of the PM have been suggested to influence signal transduction events such as the activation of phospholipase Cγ1 (PLCγ1), which has a thiol-sensitive membrane association (Kanner et al. 1992). The heterogeneous complex which has received much attention in T cell activation has been that of the TCR molecule (α/β chains) and the CD3 complex, which consists of multiple polypeptide chains (γ/δ/ε/ζ). Surface expression of the disulfide-linked homodimer of CD3ζ (CD247) is especially sensitive to oxidative stress, and it is the main signaling peptide of the CD3 complex via the tyrosine kinase ZAP-70. CD4 (monomer or disulfide homodimer) or CD8 (a disulfide heterodimer) also is closely associated with this complex within a "lipid raft" (a plasma membrane region with a higher concentration of cholesterol, sphingolipids, and saturated lipids), in that anti-CD4 or anti-CD8 have been shown to provide substantial proliferative signals only when cross-linked with antibodies to the TCR/CD3 complex. The conformation of the protein transmembrane domains of the TCR/CD3/CD4 or CD3 complex affects their association with intramembrane enzymes and the lipid-lipid domains. CD4 and CD3, which during activation are intimately associated with

TCR, usually undergo phosphorylation and internalization after T cell activation, due to their proximity to specific transmembranal and/or cytoplasmic kinases. These topographical changes with T cell activation are dependent on cellular thiols.

Protein functions can be altered by interconversions of their sulfhydryls (−SH), intra-, or interchain disulfides (−S-S-) with each other. A good example is the surface expression of CD4, in that the disulfide-linked CD4 homodimer signals better than the monomer (Maekawa et al. 2006). It also has been suggested that lck, another tyrosine kinase involved in T cell activation, is associated with thiols on the cytoplasmic C-terminal end of CD4. Thus, T cell activation involves numerous protein-protein interactions that can be influenced by redox modifications of cellular thiols. Domain effects dependent on the structure and location of costimulatory molecules, such as CD4 and CD3ζ, may influence the preferential activation of a T cell subset, in that there have been reports that Th1 and Th2 cells require the expression of distinct costimulatory signals from molecules in addition to class II-bound peptide on APCs (Weaver et al. 1988). CD30 expression by T cells was thought to be a specific marker of Th2 cells, but recent evidence suggests it is expressed by Th1 and Th2 cells but is expressed longer by Th2 cells. When released as soluble (s)CD30 during inflammation/oxidative stress, sCD30 affects the Th1/Th2 balance by binding to CD30, which is a receptor for Trx1. CD30 interaction with CD30L (CD154) leads to enhancement of Th1 cell activity. However, sCD30 blocks CD30/CD154 and affects the oxidoreductase activity of Trx1, which can affect the level of IL-12 (a disulfide-linked heterodimer of p40 and p35), the promoter of Th1 development, and p40, which blocks IL-12 activity. Thus, sCD30 can promote Th2 activity indirectly via redox effects on cytokine expression (Contasta et al. 2010). Th1 and Th2 cells also differ with regard to galectin-9 binding, which preferentially causes apoptosis of Th1 cells and enhances Th2 chemotaxis. These differences also relate to redox effects, in that galectin-9 binds to cell surface protein disulfide isomerase (PDI), a protein that promotes sulfhydryl-disulfide interchanges (Bi et al. 2011). It is surface expression of PDI that aids HIV infection of CD4 T cells. As with antioxidant changes during oxidative stress, not all immune activities uniformly undergo changes with age, in that $CD8^+$ T cells decline more rapidly than $CD4^+$ T cells, and Th2 cells become hyperactive in some older individuals. It is interesting to note that HIV-seropositive individuals progress to acquired immunodeficiency syndrome (AIDS) more readily when IL-4 levels (produced by Th2 cells) predominate over IL-2 (produced by Th1 cells). Within the mouse (M) AIDS model system, upregulation of Th2 activity also occurs. Interestingly, the activity and survival of regulatory T (Treg) cells are less sensitive to oxidative stress than effector T cell subsets due to their expression of Trx1 (Mougiakakos et al. 2011). The described differences in T cell subset sensitivities highlight the fact that many aspects of redox processes remain unresolved and many of the redox processes at the cell surface and intracellular affect immunity and involve protein and nonprotein antioxidants.

Oxidant/Antioxidant Effects on APC Modulation of the Th1/Th2 Balance

Oxidative stress on APC (macrophages (Mp) and possibly dendritic cells (DC)) allows Th2 development to predominate. This outcome may involve Trx1 effects on the level of IL-12 and p40, as discussed earlier, as well as a differential stimulation potential by APC dependent on their amounts of GSH. APC that preferentially activate Th1 and Th2 cells have been referred to as classically activate Mp, M1 cells, or redMp (Mp with a higher reductive capacity than oxMp) and alternatively activated Mp, M2, or oxMp, respectively. The differential redox capacities of redMp and oxMp are related to the higher amount of GSH in the redMp (Murata et al. 2002). IL-4, a cytokine promoting Th2 activities, can lower the GSH level of Mp, thus enhancing the number of oxMp. These GSH influences on Mp further implicate thiol chemistry in

immunomodulation. It is not unexpected that Mp would be more resistant to oxidative stress than lymphocytes since they produce oxidants. Many accessory characteristics of monocytes (production of IL-1, IL-6, and TNFα and expression of ICAM-1, LFA-3, and MHC class II) from healthy older humans do not change with age although the T cells from the elderly respond less well to PHA and the monocytes from the elderly provided less help to young T cells in response to PHA. Thus, the lack of monocyte aid may relate more to redox than cytokine assistance. Th2 cells seem to be more dependent on factors such as IL-1, which appears to be in agreement with inflammatory Mp having less GSH and being better able to activate Th2 cells. Similar to the influence of GSH on the Th1/Th2 balance controlled by Mp, the GSH levels of DC affect Th1 versus Th2 activation; however, with DC, the balance is affected by IL-27 (Kamide et al. 2011). IL-27 preferentially promotes development of Th1 cells, and like IL-12, IL-27 is a disulfide-linked heterodimer of Epstein-Barr virus (EBV)-induced gene-3 (EBI-3) and p28 (IL-30), which resemble p40 and p35 of IL-12, respectively. IL-23 is another disulfide-linked heterodimer composed of p40 (IL-23 and IL-12 share the same molecule) and p19, which resembles the p35 of IL-12. IL-23 affects development of a third $CD4^+$ effector T cell (Th17). As for IL-12, IL-23 activity is inhibited by p40. Thus, redox modifications can directly affect immune cells or indirectly affect immune cell development and function by altering the structure of regulatory cytokines.

Thiols and Aging: Early Signal Transduction Events

As mentioned earlier, antioxidants function differently in different cellular compartments. Furthermore, even at a particular location, not all antioxidants may function similarly. Even when the GSH level is lowered with the inhibition of γ-glutamylcysteine synthesis by L-buthionine-S,R-sulfoximine (BSO, a specific γ-glutamylcysteine ligase inhibitor), 2-mercaptoethanol (2ME, a potent reducing agent) is unable to support concanavalin A (ConA)- or phytohemagglutinin (PHA)-induced proliferation of human T cells. Under atmospheric oxygen conditions, 2ME is required for proliferation of mouse, but not human, lymphocytes. Mouse lymphocytes have less GSH than human lymphocytes, and mouse and human lymphocytes have other known thiol-related differences, e.g., mouse cells contain 50 % of the γ-glutamyl transpeptidase activity of human lymphocytes. In addition, ConA stimulation of human lymphocytes increases the transpeptidase activity, while the activity within mouse lymphocytes further declines.

The loss of proliferative ability with aging does not always correlate with a loss in the ability to produce IL-2. Although in vitro activation of T cells from the elderly generally results in less IL-2 production in comparison to T cells from young individuals, this deficit can be overcome by pre-culture of the peripheral blood mononuclear cells (PBMC), boosting with a vaccine, or hormone treatment. However, overall activation as measured by proliferation (DNA synthesis) usually remains depressed with PBMC from the elderly. It is interesting to note that as posited for the depressed lymphoproliferative response of PBMC from older donors, the lymphocytes from human immunodeficiency virus (HIV)-infected individuals have an elevated level of oxidative stress, in terms of lower amounts of GSH and other antioxidants. The additive oxidative effects of aging and HIV infection could be an explanation for why the onset of AIDS in older individuals appears to be more rapid. Non-HIV-infected "young" T lymphocytes depleted (90 %) of GSH by BSO also are unable to synthesize DNA (progress through S-phase) after ConA stimulation; however, cellular activation events are not inhibited in these GSH-deficient cells, in that these cells can express IL-2, IL-2R, and transferring receptor. The growth-promoting factor originally derived from adult thymic leukemic cells referred to as ADF is able to induce formation of the high-affinity IL-2 receptor (α/β/γ, CD25/CD122/CD132), and ADF is Trx1.

Hence, T cell proliferation is intimately dependent on GSH and Trx1, which likely relates to the cellular compartmentalization differences among oxidant and antioxidant processes. Oxidative stress (or aging) may modify early signal transduction events as well as later events required for proliferation. Several enzymes with immunomodulatory potential are thiol-regulated, including adenylate cyclase, 5-nucleotidase, insulin receptor, and Na^+/K^+-ATPase. Changes in the transport functions start early in G1 (Na^+, K^+, and Ca^{++} changes occur within 1 min; glucose by 10 min; amino acids and nucleosides by 1 h). Since cell transport mechanisms directly utilize thiol-sensitive proteins, thiol modulation is suggested to have regulatory influence on multiple early events involved in lymphocyte activation. As might be anticipated, many of the above activities are altered with aging. Na^+/K^+-ATPase activity decreases in lymphocytes from the elderly; membrane potential changes are less responsive with aged cells; and nucleotide pools, kinase activity, protein phosphorylation patterns, and G protein involvements have been reported to be modified with age. Many of these processes are interconnected in the activation of T cells.

GSH and Lymphocyte Activation

GSH is critical in maintaining a viable cellular redox state, which becomes modified during lymphocyte activation and proliferation. GSH (and total thiol levels) have been shown to rise significantly following lymphocyte activation by mitogens. Human lymphocytes significantly increase their GSH concentration within 4 h of activation. By 8 h after activation, there is a significant increase in surface thiols (Lawrence et al. 1996). Interestingly, when GSH production is blocked, lymphocytes can progress to G1b but cannot synthesize DNA. DNA synthesis was previously shown to be sensitive to thiol modulation. Some possible explanations for the block in DNA synthesis include interference in the glutaredoxin/thioredoxin-mediated conversion of ribonucleotide reductase, inactivation of DNA polymerase I, which is thiol-sensitive, or alteration of PCNA activity or mitosis-promoting factor. It is important to note that lymphocyte treatment with maleimides (or benzoquinone), which alkylate thiols, prevents IL-2 synthesis; however, GSH depletion does not inhibit IL-2 production, but the cells cannot proliferate due to inhibition of DNA synthesis. Cellular redox status controls production and activity of cellular transcription factors. Intracellular cysteine is required for synthesis of GSH. DNA synthesis and cell proliferation in mitogenically stimulated T lymphocytes have been shown to be upregulated with increases in extracellular cysteine and downregulated with increases in extracellular glutamate. Lymphocytes are incapable of synthesizing cysteine, because they lack a cystathionine pathway; therefore, they rely on intracellular transport of cysteine or cystine. Intracellularly, cystine must be converted to cysteine. Cysteine transport occurs via the well-characterized Na^+-dependent ASC system, whose activity increases in mitogen-stimulated lymphocytes. The less understood membrane active transport system xc is involved in cystine uptake, in which intracellular glutamate is exchanged for extracellular cystine. Surprisingly, dehydroascorbate (DHA), the oxidized product of the antioxidant vitamin C (ascorbic acid), is a more potent inducer of GSH than N-acetylcysteine, which apparently involves the pentose phosphate pathway (Perl et al. 2002).

Membranes, Aging, ROS, and Antioxidants

In addition to a direct thiol influence on surface protein conformation, modulation of surface proteins could be influenced by the lipid architecture of the membrane, including the mitochondrial membrane. Lipid domains exist within biological membranes, and aggregates of lipids may diffuse as units of compartmentalized lipid only domains or together with protein constituents with which they are specifically associated. Modification of

membrane lipid components is well known to produce alterations in cellular functions. It has been established that the inflammatory process can generate ROS by both neutrophils and monocytes/macrophages. The formation of ROS (O_2^-, H_2O_2 and $^{\cdot}OH$) can induce lipid peroxidation of the cell membrane. In addition, the superoxide anion (O_2^-) can be converted by superoxide dismutase or spontaneous dismutation to hydrogen peroxide (H_2O_2), which freely diffuses into cell membranes. H_2O_2 can oxidize (enzymatically and nonenzymatically) cellular thiols (e.g., GSH) and lead to disulfide exchange reactions between oxidized glutathione (GSSG) and protein thiols. In addition, H_2O_2 can undergo further reduction to $^{\cdot}OH$, a Fe^{++}- or Cu^{+}-dependent process. The $^{\cdot}OH$ is extremely reactive and oxidizes the nearest protein, carbohydrate, or lipid. If the reaction occurs with proteins or carbohydrates, the "damage" is limited to the particular molecule with which the $^{\cdot}OH$ reacts. However, if $^{\cdot}OH$ oxidizes a lipid molecule, a process referred to as lipid peroxidation, an autocatalytic reaction, may ensue and lead to extensive membrane alterations. The question arises as to whether this increase in ROS preferentially affects lymphocyte subsets resulting in an imbalance in "normal" immunoregulation. Intracellular endogenous oxidants such as malondialdehyde also are generated during prostaglandin synthesis from transiently produced prostaglandins PGG_2/PGH_2 and, if unregulated, may oxidize protein amino groups directly or GSH indirectly. In the presence of exogenous oxidants such as O_2^- and $^{\cdot}OH$, autocatalytic lipid peroxidation through prostaglandin endoperoxides and/or oxidation of polyunsaturated lipids can lead to PM failure or disruption of the lipid structure surrounding regulatory proteins. When lymphocytes are activated, there is generally an increase in the concentration of polyunsaturated lipids within the membrane, which accounts in part for the increase in membrane fluidity. The increase in membrane fluidity is only transient likely due to the breakdown (peroxidation) of the polyunsaturated lipids. If the basal level of oxidative stress increases (as posited in the aging process), the polyunsaturated lipid content of the membrane will decline leading to a lower state of membrane fluidity. Membrane fluidity is known to decrease with loss of unsaturated lipids. This appears to occur with aging, which may be due to loss of desaturase activities. Based on numerous criteria – electron spin resonance (ESR), fluorescence polarization, and lipid insertion studies – the membranes of cells from older individuals have less axial rotation of phospholipids and higher packing/stacking of lipids, which are indices of less fluidity.

The mitochondrial membrane and mitochondria functions are of special importance, because in the process of producing adenosine triphosphate (ATP), the electron transport chain (ETC.) of five complexes utilized to produce ATP from adenosine diphosphate (ADP) generates superoxide anion. Mitochondria efficiency is assessed as the amount of protons needed to produce ATP by ATP synthase. Since ETC. activities generate superoxide anion, mitochondrial structures are vulnerable to oxidation, which is why they contain their own superoxide dismutase (MnSOD). However, generation of H_2O_2 by MnSOD or cytoplasmic Cu/ZnSOD can be dangerous to the mitochondria, in that they contain a high content of iron, which can convert H_2O_2 to the highly reactive hydroxyl radical. Mitochondria have Trx2 and peroxiredoxins to assist in controlling the level of H_2O_2. Mitochondria are highly compartmentalized with a double membrane normally restricting influx and efflux of factors, especially oxidants generated by the oxidative phosphorylation processes and molecules associated with apoptosis. Mitochondrial DNA would be especially vulnerable to the loss of control over the level of oxidants and antioxidants. The role of mitochondria in oxidative processes, aging, and cell survival are intimately associated (Wallace 2005).

Conclusion

The ability of antioxidants to control lymphocyte activation processes and the excesses of oxidants that can arise during inflammation are critical for

maintenance of a healthy state. It is often difficult to know whether the malfunctioning of antioxidants induces an immunopathology or whether an immunopathology causes a loss in the redox balance. In either case, it is clear that the extracellular and intracellular activities of antioxidants regulate immune functions and that an imbalance of redox activities occurs with loss of lymphocytes as HIV infection progresses to AIDS and with a loss of immunoregulation as oxidative stress leads to tissue damage with type 1 diabetes, lupus, and rheumatoid arthritis. The compartmentalized redox reactions evolved to allow for the differential subcellular organelle functions needed for cell activation, cell division, and cell function. The differential immune cell sensitivities to oxidants and antioxidant modulations exist as a means for controlling the type and duration of an immune response.

Cross-References

- ▶ Autoinflammatory Diseases
- ▶ Nitric Oxide
- ▶ Prostaglandins, Leukotrienes, and Related Compounds
- ▶ Resolution of Inflammation

References

Ben-Yehuda A, Weksler ME. Host resistance and the immune system. Clin Geriatr Med. 1992;8:701–11.

Bergman WL, Dressler V, Haest CWM, Deuticke B. Cross-linking of SH-groups in the erythrocyte membrane enhances transbilayer reorientation of phospholipids. Biochem Biophys Acta. 1984;769:390–8.

Bi S, Hong PW, Lee B, Baum LG. Galectin-9 binding to cell surface protein disulfide isomerase regulates the redox environment to enhance T-cell migration and HIV entry. Proc Natl Acad Sci. 2011;2010(108):10650–5.

Contasta I, Totaro R, Berghella AM, Pellegrini P, Del Beato T, Carolei A, Adorno D. Soluble CD30: a biomarker for evaluating the clinical risk versus benefit of IFNbeta1A treatment in multiple sclerosis patients. Int J Immunopathol Pharmacol. 2010;23:213–26.

Go Y-M, Jones DP. Redox compartmentalization in eukaryotic cells. Biochem Biophy Acta. 2008;1780:1273–90.

Harman D. Free radical theory of aging: an update: increasing the functional life span. Ann N Y Acad Sci. 2006;1067:10–21.

Hu ML, Tappel AL. Glutathione and antioxidants protect microsomes against lipid peroxidation and enzyme inactivation. Lipids. 1992;27:42–5.

Kamide Y, Utsugi M, Dobashi K, Ono A, Ishizuka T, Hisada T, Koga Y, Uno K, Hamuro J, Mori M. Intracellular glutathione redox status in human dendritic cells regulates IL-27 production and T-cell polarization. Allergy. 2011;66:1183–92.

Kanner SB, Kavanagh TJ, Grossmann A, Hu S-K, Bolen JB, Rabinovitch PS, Ledbetter JA. Sulfhydryl oxidation down-regulates T-cell signaling and inhibits tyrosine phosphorylation of phospholipase Cγ1. Proc Nat Acad Sci. 1992;89:300–4.

Lawrence DA, Song R, Weber P. Surface thiols of human lymphocytes and their changes after in vitro and in vivo activation. J Leukoc Biol. 1996;60:611–8.

Maekawa A, Schmidt B, Fazekas de St. Groth B, Sanejouand Y-H, Hogg PJ. Evidence for a domain-swapped CD4 dimer as the coreceptor for binding to class II MHC. J Immunol. 2006;176:6873–6878.

McIntyre TM, Hazen SL. Lipid oxidation and cardiovascular disease: introduction to a review series. Circ Res. 2010;107:1167–9.

Mougiakakos D, Johansson CC, Jitschin R, Bottcher M, Kiessling R. Increased thioredoxin-1 production in human naturally occurring regulatory T cells confers enhanced tolerance to oxidative stress. Blood. 2011;2011(117):857–61.

Murasko DM, Goonewardene IM. T cell function in aging: mechanism of decline. Annu Rev Gerontol Geriatr. 1990;10:71–96.

Murata Y, Shimamura T, Hamuro J. The polarization of T(h)1/T(h)2 balance is dependent on the intracellular thiol redox status of macrophages due to the distinctive cytokine production. Int Immunol. 2002;14:201–12.

Perl A, Gergely P, Puskas F, Banki K. Metabolic switches of T cell activation and apoptosis. Antioxidants Redox Signal. 2002;2002(4):427–43.

Schacter D, Abbott RE, Cogan U, Flamm M. Lipid fluidity of the individual hemileaflets of human erythrocyte membranes. Ann NY Acad Sci. 1983;414:19–28.

Sohal RS, Orr WC. Relationship between antioxidants, prooxidants and the aging process. Ann NY Acad Sci. 1992;663:74–84.

Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Ann Rev Gent. 2005;39:359–407.

Weaver CT, Haurylowicz RM, Unanue ER. T helper cell subsets require the expression of distinct costimulatory signals by APC. Proc Natl Acad Sci USA. 1988;85:8181–5.

Anti-phospholipid Antibody Mechanisms of Thrombosis

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Synonyms

Autoimmune thrombosis

Definition

Anti-phospholipid antibodies (aPL) induce a prothrombotic diathesis, ultimately leading to clotting events through three main mechanisms: interference with coagulation proteins in the fluid phase, interaction with the cell types involved in the haemostatic balance, and complement activation. Placental thrombosis contributes to the pathogenesis of aPL-induced pregnancy complications together with defective placentation, decidual inflammation, and complement activation.

Introduction

Anti-phospholipid antibody syndrome (APS) is characterized by recurrent arterial and/or venous thrombosis and/or pregnancy morbidity associated with aPL detectable by anti-cardiolipin (aCL) and/or anti-β2 glycoprotein I (β2GPI) and/or lupus anticoagulant (LA) assays (Miyakis et al. 2006).

aPL are not only diagnostic autoantibodies but are believed to play a pathogenic role mediating several clinical manifestations of the syndrome. The persistent presence of aPL is a risk factor for developing the syndrome but an additional second hit is thought to be required for triggering the clinical manifestations (*two hit theory*) (Meroni et al. 2011).

Although APS is currently considered as a single disease, the clinical and the biological characteristics of the vascular involvement are different from those associated with the obstetrical problems. aPL-induced thrombophilic state plays a key role in the vascular events, but it cannot explain some clinical manifestations and most of the pregnancy complications. Additional aPL-mediated pathogenic mechanisms have been suggested (Meroni et al. 2011; Shoenfeld et al. 2009; Ruiz-Irastorza et al. 2010).

Pathogenesis of the Vascular Manifestations

The association between aPL and both venous and arterial thrombosis is supported by several epidemiological studies, and clot formation is the key event for vascular manifestations (Meroni et al. 2011). The thrombophilic state is supported by the aPL interference with both soluble components and different cell types involved in the coagulation cascade (Meroni et al. 2011). Table 1A and Fig. 1 report the main aPL-mediated pathogenic mechanisms that have been related to thrombosis in APS.

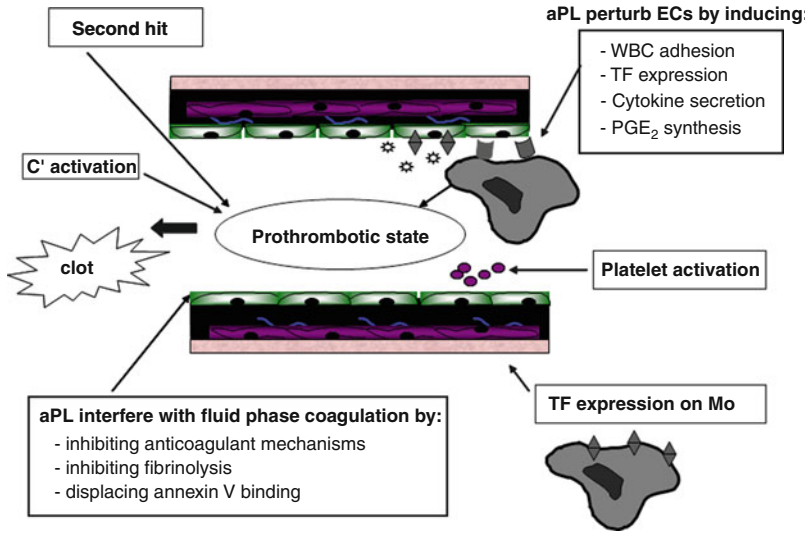
Most of the pathogenic mechanisms potentially responsible for thrombus formation have been demonstrated using in vitro models. However, three different in vivo models of thrombosis induced respectively by mechanical, chemical, or photochemical trauma have confirmed that aPL are able to increase venous and/or arterial thrombus formation in vivo (Meroni et al. 2011). Two of these experimental models demonstrated the ability of aPL to increase the thrombus size already triggered respectively by the mechanical or the chemical stimulus and to slow down its dissolution. On the other hand, the third model showed that the passive infusion of human aPL IgG together with a small amount of lipopolysaccharide (LPS) is able by itself to start clotting in the rat mesenteric arterial microcirculation (Fischetti et al. 2005).

The evidence of aPL interference with fluid phase components of coagulation has been

Anti-phospholipid Antibody Mechanisms of Thrombosis, Table 1 Anti-phospholipid antibody–mediated pathogenic mechanisms for thrombosis (A) and fetal loss (B)

(A) Thrombosis	(B) Fetal loss
Interference with fluid phase coagulation components:	Placental thrombosis
Inhibition of natural anticoagulants	Inflammatory villitis
Inhibition of Protein C activation	Inhibition of syncytium-trophoblast differentiation
Disruption of the Annexin V shield	Induction of decidual cells inflammatory phenotype
Inhibition of fibrinolysis	Embryo and/or placental apoptosis
Interference with cells of the coagulation cascade:	Complement activation
Endothelial cell perturbation	
Monocyte activation (TF expression)	
Platelet activation	
Complement activation	

provided mostly by in vitro models and by few ex vivo experiments (Giannakopoulos et al. 2007; Pierangeli et al. 2008; Meroni et al. 2011). aPL have been found to bind to some members of the serine protease (SP) family, which enlists proteins involved in hemostasis (pro-coagulant factors as thrombin, prothrombin [PT], FVIIa, FIXa, and FXa, and anticoagulants as protein C) as well as fibrinolysis (plasmin and tissue plasminogen activator [tPA]). aPL interaction with these proteins may be explained by the evidence that β2GPI and SP enzymatic domains share conformational epitopes. Most importantly, aPL interact with thrombin and FXa, interfering with the formation of thrombin-antithrombin (AT) and FXa-AT complexes, thus hindering the AT inactivation of thrombin and FXa. Moreover, a disruption of protein C and protein S pathways has been described. Indeed, aPL are reported to decrease activated protein C (APC) activity through competition



Anti-phospholipid Antibody Mechanisms of Thrombosis, Fig. 1 aPL interfere with: (A) the fluid phase coagulation factors, (B) potentiate platelet aggregation induced by other agonists, (C) induce monocytes (Mo) activation with the upregulation of Tissue Factor (TF ⧫) expression, and (D) endothelial cell (EC) perturbation with the induction of a pro-adhesive phenotype for white blood cells (WBC), TF upregulation, cytokine (✲) release and prostacyclin PGE_2 metabolism changes

with APC for PL binding. Accordingly, an increased APC resistance has been demonstrated in APS patients.

Several groups have also found decreased levels of both proteins C and S and aPL with affinity for either protein S or C in APS subjects. In addition, some aPL were shown to inhibit plasmin-mediated fibrinolysis, consistent with the evidence that IgGs from APS patients lead to an impairment of fibrin dissolution by plasmin. Moreover, some aPL subpopulations were found to bind to tPA, inhibiting tPA-mediated conversion of plasminogen to plasmin. Concordantly, antibodies against tPA have been described in APS patients, with an inverse correlation with plasma tPA activity.

Besides the inhibitory effects on anticoagulants, aPL may increase the enzymatic activity of pro-coagulants. There is evidence that some aPL subpopulations induce a gain of function of PT, leading to increased fibrin production. In addition, aPL have been demonstrated to disrupt the crystallization on endothelial cell (EC) monolayer of Annexin A5, a potent anticoagulant that prevents PL bioavailability for coagulation enzymes (Giannakopoulos et al. 2007; Pierangeli et al. 2008; Meroni et al. 2011).

More recently, research has focused on aPL interaction with cells involved in the haemostatic balance including platelets, monocytes, and ECs (Meroni et al. 2011).

Platelet Involvement

The first hint of platelet involvement in aPL-mediated thrombus formation comes from the frequent clinical finding of a mild thrombocytopenia among APS patients.

It is well described that aPL induce aggregation and activation of platelets. To note, prestimulation by agonists such as thrombin or collagen is necessary for aPL to potentiate platelet activation, as it leads to the exposure of phosphatidylserine (PS) on the cell outer membrane. Being a negatively charged PL, PS favors the adhesion of the cationic β2GPI on the platelet membrane, eventually offering the right antigenic target for aPL. aPL have been shown to neutralize β2GPI interaction with von Willebrand factor (vWF). β2GPI acts as a biologically relevant inhibitor of VWF function, thus interfering with vWF-dependent platelet adhesion. This action could contribute to aPL-induced thrombosis and may explain the consumptive thrombocytopenia frequently observed in aPL carriers. Moreover, aPL have been shown to enhance the expression of platelet membrane glycoproteins (GPs) IIb/IIIa and IIIa. GPs are fibrinogen receptors that mediate platelet aggregation, whose role in APS pathogenesis is further supported by in vivo findings. In fact, aPL did not affect thrombus formation in GPIIb/IIIa-deficient mice and pretreatment with a monoclonal anti-GPIIb/IIIa antibody inhibited aPL-mediated reduced thrombus formation. In vivo evidence of platelet activation by aPL has been gained in other models: (i) aPL produced a platelet-rich thrombus in rats, after treatment with low concentration of adenosine diphosphate (ADP), and (ii) platelet involvement was reported in the thrombus formation by photochemical injury in the rat.

Concordant findings regarding platelet activation by aPL have been provided by ex vivo studies. Elevated levels of platelet-derived thromboxane (the major eicosanoid produced by platelets, [TX]) metabolic breakdown products have been found in the urine of APS patients.

aPL interaction with platelets and the consequent activation is mediated by cell membrane receptors. To date, β2GPI has been demonstrated to bind two surface receptors on platelets. Apolipoprotein E Receptor 2′ (ApoER2′) is the only member of Low-Density Lipoprotein (LDL) receptor family expressed by platelets; its LDL-binding domain I has been shown to recognize positively charged patch of lysine residues in domain V of β2GPI. An inhibitor of LDL receptors was shown to block platelet activation and TX synthesis induced by aPL. Lastly, β2GPI has been shown to bind directly GPIba, a subunit of the GPIb-IX-V platelet receptor.

Involvement of Monocytes

The upregulation of tissue factor (TF), the major initiator of the clotting cascade, has also been advocated as an important mechanism to explain the prothrombotic aPL effects. aPL significantly increase TF expression in ECs and monocytes, IgGs from APS patients are stronger TF inducers than IgGs from aPL asymptomatic carriers.

In particular, TF expression on monocytes is increased in patients with APS and correlates with the expression of β2GPI. Accordingly, cell-surface expression of TF on monocytes is higher in APS patients than in subjects without thrombotic events. Interestingly, vascular endothelial growth factor (VEGF) stimulates TF expression in monocytes through its tyrosine-kinase receptor Flt-1 and expression of both VEGF and Flt-1 is increased in monocytes from APS patients. On monocytes, aPL have been suggested to interact with β2GPI in association with Annexin A2 and Toll-like Receptor (TLR) 4 within lipid rafts. Other authors provided indirect evidence of TLR2 involvement in mediating aPL-induced monocyte activation (Boles and Mackman 2010; Meroni et al. 2011).

Involvement of Endothelial Cells

The endothelium is a key player in the pathogenesis of APS. The vascular perturbation induced by aPL drives a pro-inflammatory and pro-coagulant endothelial phenotype, ultimately leading to clotting events.

aPL have been shown to induce a significant upregulation of cellular adhesion molecules (CAMs) such as ICAM-1, VCAM-1, and E-selectin in EC in vitro cultures. The increased expression of CAMs on the vascular surface is in turn responsible for the increased leukocyte adhesion to the endothelium, hence favoring the prothrombotic diathesis of the syndrome. Moreover, aPL promote the in vitro synthesis of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and IL-8 in ECs. Lastly, aPL modulate the vascular tone by inhibiting endothelial nitric oxide synthase and altering prostaglandin metabolism. Accordingly, in vivo studies have shown that passive infusion of aPL induced an increase in the rate of leukocytes adhering to the endothelium.

Ex vivo studies in APS subjects reported raised levels of soluble CAMs such as s-ICAM-1, VCAM-1, P-selectin although with contrasting results among the different groups. More importantly, APS patients were found to display endothelial perturbation, as suggested by the impaired brachial artery flow–mediated vasodilation responses and the significant increase in the numbers of circulating ECs and in t-PA and vWF titers (Meroni et al. 2011).

aPL-induced effects on the endothelium are mainly mediated by the reactivity of the autoantibodies with β2GPI expressed on the endothelial cell membrane. Since the evidence for an endothelial synthesis of the molecule is lacking, it has been suggested that β2GPI is adhering to EC membrane. The adhesion is apparently mediated by several different receptors whose nature remains uncertain. Annexin A2, a receptor for tPA and plasminogen, has been demonstrated to directly bind β2GPI. As Annexin A2 lacks an intracytoplasmatic tail, a co-receptor is required to trigger signaling cascade. TLR 2 and 4, Heparan sulfate, and ApoER2′ have all been indirectly shown to bind β2GPI on endothelial surface. TLRs 2 and 4 have been found to mediate also intracellular aPL signaling in ECs; to note, TLR2 is expressed by ECs only upon cell activation while TLR4 is constitutively expressed. More recently, it has been suggested that a multiprotein complex including TLR4 and Annexin A2 is involved in aPL-induced EC activation (Allen et al. 2012). Annexin A2, TLR4, and ApoER2′ have also been investigated in vivo. Animals deficient in any one of these molecules are only partially protected against aPL thrombogenic effects, suggesting redundancy in the signaling cascade (Meroni et al. 2011).

With regard to the downstream signaling pathways engaged by aPL, there is general agreement that nuclear factor κB (NFκB) and p38 mitogen-activated protein kinase (MAPK) are involved both in EC and monocyte activation (Meroni et al. 2011).

Pathogenic Antibodies

aPL are generally thought to be heterogeneous but there is evidence that only the antibodies

reacting with PL-binding proteins – in particular β2GPI and PT – can be pathogenic.

For example, aPL reacting with human β2GPI and cross-reacting with the murine, rat, and hamster molecule were shown to be pathogenic in all the in vivo models. In particular, the effect was reproduced by affinity purified anti-β2GPI IgG and specific absorption of the anti-β2GPI activity inhibited the thrombotic effect (Meroni et al. 2012). In view of these experimental findings, β2GPI-dependent aPL should be considered the main antibody subpopulation responsible for the thrombotic manifestations in the syndrome.

The analysis of the fine specificity of β2GPI-dependent aPL is still a matter of investigation. Preliminary studies showed heterogeneous activity of anti-β2GPI autoantibodies in APS patients, which are directed, often concurrently, against various (mostly linear) epitopes located in β2GPI-domain(D)1, D4, and D5. More recently, antibodies that recognize a cryptic epitope (Gly40-Arg43) in DI of β2GPI were closely associated with LA activity and strongly correlated with thrombosis and obstetrical complications. The ability of soluble DI to protect mice from the thrombogenic effect of aPL was considered a proof of concept for the key pathogenic role of anti-DI antibodies (Mahler et al. 2012). Interestingly, IgG reacting with β2GPI in sera from asymptomatic patients, individuals with leprosy, or children with atopic dermatitis have been reported to preferentially recognize epitopes on D4/5. Moreover, the ratio between anti-β2GPI-D1 and anti-β_2GPI-D4/5 IgG antibody reactivities was suggested to provide important information to discriminate between APS patients and individuals with other pathologies (Mahler et al. 2012). The D1 of β2GPI is usually hidden as a result of its linkage with D5 in the circular form of the molecule and is presented to the afferent limb of the immune system when the molecule is opened after binding to anionic PL monolayers or scavenging LPS (Agˇar et al. 2006).

Besides β2GPI, aPL are also known to react with other PL-binding proteins, in particular PT. The way anti-PT antibodies exert their procoagulant effect is unknown. In vitro studies suggested that anti-PT may perturb ECs by reacting with the molecule on the cell surface. However, there is no clear evidence for anti-PT pathogenic activity in animal models and their pathogenic role is mainly supported by the epidemiological association with thrombosis (Meroni et al. 2011). The best link was reported for antibodies detected by the PS-PT assay, suggesting that the pathogenic antibodies may recognize a conformational epitope(s) expressed by PT when complexed with anionic PL in the presence of calcium ions (Bertolaccini 2012).

The pro-coagulant mechanisms of aPL are mainly related to their ability to react with PL-binding proteins expressed on the cell membrane of different cell types. It is still not clear whether aPL react in a significant manner with the PL-binding proteins (in particular β2GPI and PT) in fluid phase. In this regard, all aPL are low-avidity antibodies, suggesting that complex formation in the fluid phase requires stoichiometric antigen-antibody ratios that are uncommon in patients (Tincani et al. 1996). This point further supports the notion that aPL reactivity with the target molecules expressed on the cell membranes plays a major role in APS pathogenesis. In particular, β2GPI is expressed on the cell membranes at high antigenic density and so more easily recognized by low-avidity autoantibodies. In addition, it has been reported that circulating β2GPI is usually present in the circular-closed form while it is opened only after binding to negative PL or after complexing with LPS. Since the autoantibodies were reported to react mainly with the opened form (i.e., reacting with the exposed cryptic epitope of D1), this finding may further explain the reason for the limited reactivity with the circulating form.

Is Clotting Also Involved in the Pathogenesis of the Pregnancy Complications?

The persistent presence of aPL represents the most frequent acquired risk factor for a treatable cause of recurrent pregnancy loss and for pregnancy complications (early and severe

pre-eclampsia) (Miyakis et al. 2006). Such an association is clearly supported by epidemiological studies and by experimental models showing that passive transfer of aPL IgG induces fetal loss and growth retardation in pregnant naive mice (Meroni et al. 2011).

Intraplacental thrombosis with impairment of maternal–fetal blood exchange was initially suggested to be the main pathogenic mechanism. Accordingly, in vitro studies showed that aPL may induce a pro-coagulant state at the placental level through different mechanisms including the ability of the aPL/anti-β2GPI to disrupt the anti-coagulant Annexin A5 shield on trophoblast and EC monolayers. The reduced distribution of Annexin A5 covering the intervillous surfaces found in placentas of aPL-positive women was thought to represent the in vivo demonstration of such a mechanism (Rand et al. 2010). These observations were not confirmed by other studies which failed to show intravascular or intervillous blood clots; histopathological findings suggestive of thrombosis cannot be detected in the majority of APS miscarriage samples and placentas (Meroni et al. 2010).

There is evidence from experimental animal models that repeated intraperitoneal injections of large amounts of human IgG with aPL activity to pregnant naive mice after embryo implantation induce marked placental inflammatory damage that results in fetal resorption and growth retardation. Immunohistochemical and histological examination of decidua showed deposition of human IgG and murine complement, neutrophil infiltration, and local tumor necrosis factor (TNF) α secretion, associated with a transient but significant increase in blood TNFα levels. Several lines of evidence support the role played by the complement system in this model, as suggested by the protection of animals deficient in complement components or following in vivo complement inhibition (Meroni et al. 2011).

In another experimental fetal loss model, mice deficient in D6 (a placental receptor which recognizes and targets to degradation most inflammatory chemokines of the CC subfamily) were more susceptible to fetal loss when passively infused with small amounts of human aPL IgG than pregnant wild-type mice or mice infused with normal IgG (Martinez de la Torre et al. 2007).

Altogether these findings strongly suggest that additional pathogenic mechanisms are present.

Such experimental data stand in contrast with the lack of evidence of: (i) acute local inflammatory events and complement deposition in abortive material or term placentae from APS women and (ii) the efficacy of corticosteroid treatment for preventing aPL-associated fetal loss (Meroni et al. 2011). Finally, an inflammatory process does not seem to be involved in another model of fetal resorption and growth retardation obtained by i.v. injection of small amount of human aPL IgG into mice before implantation, as indicated by the histological analysis of the placentae which failed to show clear signs of inflammation (Martinez de la Torre et al. 2012).

Evidence has been collected from in vitro experimental models for alternative pathogenic mechanisms supporting the ability of aPL to directly target the maternal decidua and the invading trophoblast. Table 1B reports all the pathogenic mechanisms that have been described to explain the aPL-mediated pregnancy complications.

As a whole, these findings suggest that APS-associated pregnancy complications can be mediated by different and not alternative pathogenic events which are not necessarily related to the aPL pro-coagulant effect or to inflammation. On the other hand, data from in vivo animal models as well as histological examination of human term placentae can be biased by the fact that findings are restricted to a given period of the pregnancy when the investigation was performed or at the time of autoantibody passive infusion. So, it is possible that different mechanisms can take place at different times during the pregnancy.

The expression of β2GPI on the trophoblast cell membranes is the pre-requisite to explain the aPL/anti-β2GPI placental tropism. β2GPI binds to PS exposed on the external cell membranes of trophoblast undergoing syncytium formation, but additional receptors may also be involved. Hence, β2GPI-dependent aPL appear to represent the main pathogenic autoantibodies in obstetrical APS. It has been hypothesized that most of these

potential pathogenic autoantibodies can be absorbed at the placental level (where β2GPI is expressed) and not transferred to the fetus. This would explain why thrombotic events are rarely reported in babies born to aPL-positive mothers in spite of the high thrombophilic profile of neonates (Meroni et al. 2011).

Unsolved Issues Regarding Anti-phospholipid Antibody-Induced Thrombosis

A *two hit hypothesis* has been suggested to explain the clinical observation that the thrombotic events occur only occasionally in spite of the persistent presence of aPL. The antibody (*first hit*) induces a thrombophilic condition but clotting takes place in the presence of another thrombophilic condition (*second hit*) (Meroni et al. 2011). Accordingly, the administration of small amount of LPS was required for human β2GPI-specific aPL IgG to display their thrombogenic effect in the rat mesenteric microcirculation (Fischetti et al. 2005). In line with this observation, it has been suggested that infectious processes may be the *second hit* since they frequently precede the full-blown picture of the syndrome and may be the initiator of the catastrophic subtype (Shoenfeld et al. 2006). This hypothesis fits well with the potential involvement of Pattern Recognition Receptors (i.e., TLRs) in sensing microbes and triggering an inflammatory response. Since TLR 2 and 4 have been reported to contribute to EC and/or monocyte activation by β2GPI-dependent aPL, one can speculate that the combination of the infection plus the TLR perturbation mediated by the autoantibodies overcome the threshold for triggering thrombosis (Meroni et al. 2011). Alternatively, infection/inflammation may increase the expression of the target antigen for aPL or the expression of antigenic epitopes that are hidden in resting conditions. In line with this hypothesis, preliminary data showed that LPS is able to upregulate the β2GPI expression in murine tissues (Agostinis et al. 2011).

The two hit hypothesis does not fit well with the APS obstetrical manifestations. In fact, passive infusion of IgG fractions with aPL activity induces fetal loss in naive pregnant mice without necessarily requiring a second hit. β2GPI is largely expressed in placental tissues even in physiological conditions and binding of labeled exogenous β2GPI infused into naive pregnant mice to trophoblast and EC in the labyrinth was recently documented in vivo by eXplore Optix™ imager (Agostinis et al. 2011). So, the large availability of the target antigen for potentially pathogenic aPL at the placental level is in strong contrast with the lack of a comparable expression in other tissues of naïve mice and even in highly vascularized human tissues such as kidney. It is possible to speculate that a high expression of β2GPI at the placental level together with the hormonal and blood flow modification linked to the pregnancy is sufficient to favor the pathogenic activity of the autoantibodies without any additional factor (Meroni et al. 2012).

Complement activation has been demonstrated as a necessary step in both aPL-mediated thrombosis and fetal loss. Sera from the majority of APS patients were found to fix complement in vitro. However, the strongest evidence of the role of complement in mediating aPL thrombotic events came from in vivo models. Animals deficient in complement components or complement receptors or treated with inhibitors of complement activation were protected from the aPL thrombogenic effects. However, a clear decrease of complement levels has not been described in patients, with only two studies reporting mild hypocomplementemia in primary APS (Meroni et al. 2011).

Similarly, the complement role in mediating aPL-induced pregnancy complications is supported mainly by in vivo experimental models. Pregnant mice deficient in complement C3 or C5, or C5a anaphylatoxin chemotactic receptor, or treated with an inhibitor of C3 convertase, did not experience aPL-induced fetal loss. In humans, a retrospective study found complement deposition in placenta tissues from aPL-positive women. However, a case study reported no complement deposition in fetuses miscarried by women with primary APS, whereas a more recent prospective study on full-term placentas and abortive specimens showed

only mild complement deposition but without any relationship with pregnancy outcome or therapy.

So, definite conclusions about the involvement of complement in APS-associated thrombosis and miscarriages cannot currently be drawn; however, the potential role of complement in aPL-mediated clinical manifestations should not be discounted.

Cross-References

- ▶ Antiphospholipid Syndrome Treatment
- ▶ Antiphospholipid Syndrome, Clinical Manifestations

References

Agˇar C, Gwendolyn MA, van Os GMA, Matthias Mörgelin M, Richard R, Sprenger RR, Marquart JA, Urbanus RT, Ronald HWM, Derksen RHWM, Joost CM, Meijers JCM, de Groot PG. β2-Glycoprotein I can exist in 2 conformations: implications for our understanding of the antiphospholipid syndrome. Blood. 2006;116:1336–43.

Agostinis C, Biffi S, Garrovo C, Durigutto P, Lorenzon A, Bek A, Bulla R, Grossi C, Borghi MO, Meroni P, Tedesco F. In vivo distribution of β2 glycoprotein I under various pathophysiologic conditions. Blood. 2011;118:4231–8.

Allen KL, Fonseca FV, Betapudi V, Willard B, Zhang J, McCrae KR. A novel pathway for human endothelial cell activation by antiphospholipid/anti-β2 glycoprotein I antibodies. Blood. 2012;119:884–93.

Bertolaccini ML. Antibodies to prothrombin. Lupus. 2012;21:729–31.

Boles J, Mackman N. Role of tissue factor in thrombosis in antiphospholipid antibody syndrome. Lupus. 2010;19:370–8.

Fischetti F, Durigutto P, Pellis V, Debeus A, Macor P, Bulla R, Bossi F, Ziller F, Sblattero D, Meroni P, Tedesco F. Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor. Blood. 2005;106:2340–6.

Giannakopoulos B, Passam F, Rahgozar S, Krilis SA. Current concepts on the pathogenesis of the antiphospholipid syndrome. Blood. 2007;109:422–30.

Mahler M, Norman GL, Meroni PL, Khamashta M. Autoantibodies to domain 1 of beta 2 glycoprotein 1: A promising candidate biomarker for risk management in antiphospholipid syndrome. Autoimmun Rev. 2012;12:313–7.

Martinez de la Torre Y, Locati M, Buracchi C, Dupor J, Cook DN, Bonecchi R, Nebuloni M, Rukavina D, Vago L, Vecchi A, Lira SA, Mantovani A. Protection against inflammation- and autoantibody-caused fetal loss by the chemokine decoy receptor D6. Proc Natl Acad Sci USA. 2007;104:2319–24.

Martinez de la Torre Y, Pregnolato F, D'Amelio F, Grossi C, Di Simone N, Pasqualini F, Nebuloni M, Chen P, Pierangeli S, Bassani N, Ambrogi F, Borghi MO, Vecchi A, Locati M, Meroni PL. Anti-phospholipid induced murine fetal loss: novel protective effect of a peptide targeting the β2 glycoprotein I phospholipid-binding site. Implications for human fetal loss. J Autoimmun. 2012;38:209–15.

Meroni PL, Tedesco F, Locati M, Vecchi A, Di Simone N, Acaia B, Pierangeli SS, Borghi MO. Anti-phospholipid antibody mediated fetal loss: still an open question from a pathogenic point of view. Lupus. 2010;19:453–6.

Meroni PL, Borghi M, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol. 2011;7:330–9.

Meroni PL, Raschi E, Grossi C, Pregnolato F, Trespidi L, Acaia B. Borghi MO Obstetric and vascular APS: same autoantibodies but different diseases? Lupus. 2012;21:708–10.

Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, Groot DE, Koike T, Meroni PL, Reber G, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295–306.

Pierangeli SS, Chen PP, Raschi E, Scurati S, Grossi C, Borghi MO, Palomo I, Harris EN, Meroni PL. Antiphospholipid antibodies and the antiphospholipid syndrome: pathogenic mechanisms. Semin Thromb Hemost. 2008;34:236–50.

Rand JH, Wu XX, Quinn AS, Taatjes DJ. The annexin A5-mediated pathogenic mechanism in the antiphospholipid syndrome: role in pregnancy losses and thrombosis. Lupus. 2010;19:60–9.

Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. Lancet. 2010;376:1498–509.

Shoenfeld Y, Blank M, Cervera R, Font J, Raschi E, Meroni PL. Infectious origin of the antiphospholipid syndrome. Ann Rheum Dis. 2006;65:2–6.

Shoenfeld Y, Meroni PL, Toubi E. Antiphospholipid syndrome and systemic lupus erythematosus: are they separate entities or just clinical presentations on the same scale? Curr Opin Rheumatol. 2009;21:495–500.

Tincani A, Spatola L, Prati E, Allegri F, Ferremi P, Cattaneo R, Meroni P, Balestrieri G. The anti-beta2-glycoprotein I activity in human anti-phospholipid syndrome sera is due to monoreactive low-affinity autoantibodies directed to epitopes located on native beta2-glycoprotein I and preserved during species' evolution. J Immunol. 1996;157:5732–8.

Antiphospholipid Syndrome Treatment

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Synonyms

Hughes Syndrome

Definition

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by recurrent thrombotic events and pregnancy morbidity, in the presence of distinct autoantibodies. The treatment of APS should be directed to subgroups based on clinical manifestations, immunological findings, and whether accompanied by other autoimmune disease. The treatment includes anticoagulants and/or aspirin.

Treatment for Thrombosis

Recently updated criteria for APS in 2006 define the syndrome as the combination of a clinical event (including at least one thromboembolic event, pregnancy morbidity, or both) and an abnormal test result (including the presence of antibodies to cardiolipin (aCL) or beta-2-glycoprotein I (anti-b2GPI) or the presence of the lupus anticoagulant (LAC) detectable at least twice over an interval of 12 weeks or more) (Miyakis et al. 2006). Thromboses are usually recurrent and frequently involve the same territory in subsequent thrombotic events (Cervera et al. 2002). Updated treatment options are presented according to clinical and antibody status (Erkan and Pierangeli; Galli et al. 2003; Prandoni et al. 2009; Cosmi et al. 2010; Ramires de Jesus et al. 2012; Heilman et al. 2011; Bramham et al. 2010; Ruiz-Irastorza et al. 2010; Danza et al. 2012; Cohen et al. 2010; Branch 2011; Ruiz-Irastorza et al. 2011; Scoble et al. 2011).

1. *Antibodies and risk of thrombosis*: The risk of developing APS and thrombotic events is directly related to the number and titers of antiphospholipid antibodies (aCL, anti-b2-GPI, and LAC). LAC is linked most strongly with thrombotic events (Erkan and Pierangeli; Galli et al. 2003). LAC increases the risk for stroke by a factor of 46, and for myocardial infarct by a factor of 11. This risk is far less for individuals with elevated anti-b2GPI titers b2-GPI which is only associated with stroke. In SLE, triple positivity (aCL, anti-b2GPI, LAC) confers an increased risk of thrombotic events. In addition, SLE patients with persistently elevated titers of aCL are at increased risk for thrombosis. APS patients with conventional cardiovascular risk factors (smoking, hypertension, high cholesterol levels, and use of oral contraceptives) are at increased risk for thrombosis. A lower risk of thrombosis is observed among individuals with intermittent low–medium titers of aCL or anti-b2-GPI. The presence of multiple positive antiphospholipid antibodies may also be associated with pregnancy morbidity.
2. *Treatment for the first venous thromboembolic event (VTE)*: Effective standard anticoagulation for VTE with vitamin K antagonists for venous thromboembolism (VTE) is recommended with a goal INR of 2 to 3. Higher intensity therapy may be associated with bleeding or paradoxical thrombotic events in APS patients. For the first event, treatment should be for 3–6 months if there was a provoked VTE (such as surgery or trauma), single positivity with aCL or anti-b2GPI, and primary APS. Before discontinuation of therapy, repeated

ultrasound-Doppler and D-dimer levels are recommended to assess residual thrombosis (Prandoni et al. 2009; Cosmi et al. 2010).

Long-term therapy after a first event may be appropriate for patients with unprovoked VTE, triple positivity, the development of pulmonary embolus, those with concomitant thrombophilia, and patients with associated autoimmune diseases (Erkan and Pierangeli).

3. *Treatment for recurrent VTE*: Indefinite antithrombotic treatment is warranted for individuals with APS and recurrent VTE. Strict control of cardiovascular risk factors is recommended for high-risk aPL profile individuals to reduce the risk of an event. Patients with definite APS and recurrent events despite warfarin with a target intensity of INR 2.0–3.0 should receive indefinite therapy with warfarin with a target intensity of INR 3.0–4.0 or an extended therapeutic dose of low molecular weight heparin (LMWH) (Erkan and Pierangeli).
4. *Treatment for first arterial thromboembolic event (ATE)*: Although VTE is usually the presenting event, patients have numerically more arterial embolic events. Patients should receive intensive anticoagulant therapy with an aim of INR 3.0–4.0 or combined anticoagulant (INR: 2.0–3.0) and antiplatelet therapy (e.g., low-dose aspirin) following a bleeding risk assessment. Non-SLE patients with a first non-cardioembolic cerebral stroke, a low-risk aPL antibody status, and reversible precipitating factors may receive antiplatelet therapy alone. Patients with definite APS and recurrent events despite warfarin with a target intensity of INR 2.0–3.0 should receive indefinite therapy with warfarin with a target intensity of INR 3.0–4.0 or extended therapeutic dose of LMWH (Erkan and Pierangeli).
5. *Treatment for antibody carriers without clinical disease*: Although not based on strong evidence, it is suggested that non-SLE patients that are high-risk aPL carriers should receive primary prevention with indefinite low-dose aspirin (Ruiz-Irastorza et al. 2011). There is good evidence that carriers of antiphospholipid antibodies (without clinical disease) should have thromboembolic prophylaxis for high-risk situations such as surgery, immobilization, and puerperium (Ruiz-Irastorza et al. 2011). The preferred treatment is LMWH. Strict control of cardiovascular risk factors is recommended for high-risk aPL profile individuals to reduce the risk of thrombosis (Erkan and Pierangeli).
6. *Treatment for antibody carriers with SLE but without APS clinical disease*: It is suggested that SLE patients that are carriers of LAC or persistent medium–high titers of aCL receive prophylaxis with hydroxychloroquine (Plaquenil) and low-dose aspirin (Ruiz-Irastorza et al. 2011).

Treatment for Obstetric APS

In obstetric APS, there are maternal and fetal morbidities. The most common maternal complications are preeclampsia, eclampsia, and placental insufficiency. Fetal morbidities are fetal compromise (first and second trimester) and premature birth. Both miscarriage and maternal thrombosis may occur simultaneously in 2.5–5 % of cases.

Recurrent miscarriage occurs in approximately 1 % of women of the reproductive age. In APS, some 10–15 % of women will experience recurrent miscarriages. Antiphospholipid antibodies are encountered in 5–20 % of women with recurrent miscarriages. About one third of untreated women with APS will develop preeclampsia during pregnancy, and more than 10 % will deliver infants that are small for gestational age.

All pregnant women with SLE or other autoimmune diseases should be tested for antiphospholipid antibodies and LAC (Ramires de Jesus et al. 2012; Heilman et al. 2011; Bramham et al. 2010; Ruiz-Irastorza et al. 2010; Danza et al. 2012).

7. *APS and pregnancy*: Pregnant women with APS have the likelihood of live births of up to 80 % if treated, but only 15 % if untreated. Pregnant women with APS should attend a high-risk pregnancy clinic every 4 weeks until mid-pregnancy, then every 1–2 weeks

until delivery. At these visits, blood pressure, urine spot checks, or a 24-h urine collection for proteinuria should be performed. Physical exam for thrombosis and obstetric ultrasound and Doppler every 4 weeks starting at 18–20 weeks are warranted. Patients should be aware of symptoms of preeclampsia (Ramires de Jesus et al. 2012; Heilman et al. 2011). Anticoagulation should be initiated when a live embryo is detected by ultrasound (Ramires de Jesus et al. 2012).

8. *Antibodies and risk of fetal morbidity*: The risk of developing APS and fetal morbidity is directly related to the type of aPL antibodies (aCl, anti-b2-GPI, and LAC). LAC is strongly correlated with recurrent fetal losses before the 24th week of gestation. Its effect on recurrent early miscarriages (REMs) is not clear. LAC most strongly influences pregnancy outcome (Danza et al. 2012). In addition, patients with persistently elevated titers of aCL IGG or IGM are at increased risk for recurrent fetal losses before the 24th week of gestation. aCL IGG is also associated with REM, but anti-b2-GPI is not. Elevated titers of aCL IgM are associated with late recurrent fetal losses. Triple positivity is associated with poor pregnancy and fetal outcomes. Newborns of APS mothers with triple positivity have a higher rate of complications including respiratory distress, infections, and bronchopulmonary dysplasia. Women with pregnancy morbidity only (without a history of thrombosis) and given conventional therapy tend to have better neonatal outcomes (Cohen et al. 2010; Branch 2011).
9. *Recurrent early miscarriage (REM)*: According to the Cochrane systematic review, treatment to reduce the risk of REM should include unfractionated heparin (5,000 U twice daily). However, expert opinion suggests treatment with 5,000–7,500 U of heparin or LMWH twice daily with low-dose aspirin (Cohen et al. 2010; Branch 2011).
10. *Fetal death*: To reduce the risk of fetal death, treatment with aspirin and unfractionated heparin 5,000 U twice daily or LMWH in prophylactic doses are the recommendations (Cohen et al. 2010; Branch 2011).
11. *Severe preeclampsia or placental insufficiency (delivery < 34 weeks)*: Treatment with aspirin and unfractionated heparin in a prophylactic or intermediate dose is suggested to reduce the risk of preeclampsia or placental insufficiency (Cohen et al. 2010; Branch 2011).
12. *Postpartum care*: The main recommendation is to continue the same medication protocol given during pregnancy. Most experts agree that treatment should continue for about 6 weeks postpartum. The therapeutic options are low-dose aspirin, heparin, or LMWH.

    Of note, low-dose aspirin, heparin, warfarin, and hydroxychloroquine are considered acceptably safe during lactation (Cohen et al. 2010; Branch 2011).
13. *Long-term implications in obstetric APS only*: Treatment with aspirin and LMWH during pregnancy will improve gestational outcomes, but not all obstetric morbidities will be reduced. If the patient had pregnancy issues only, long-term low-dose aspirin is recommended (Cohen et al. 2010; Branch 2011).
14. *Long-term implications in obstetric and thrombotic APS*: Pregnant women with APS who have experienced thrombosis have worse outcomes. They have more preterm deliveries and eclampsia and deliver more babies that are small for gestational age as compared with otherwise similar women with APS who do not have a history of thrombosis (Ramires de Jesus et al. 2012). Treatment consists of low-dose aspirin and full anticoagulant doses of heparin. Warfarin may be used after 6–12 weeks of pregnancy. Heparin can be switched to warfarin from 14 to 36 weeks pregnancy (INR 2–3 for first events; 3–4 for recurrent events). Warfarin can be reinstituted following pregnancy. Heparin can be discontinued and renewed 12 h before and after a procedure such as epidural

anesthesia. Low-dose aspirin can be continued through labor though some experts recommend discontinuing it 1 week prior to labor. If the patient had previous thrombosis, especially an arterial event such as stroke and pregnancy complications, anticoagulation for life is necessary (Bramham et al. 2010; Cohen et al. 2010; Branch 2011; Ruiz-Irastorza et al. 2011).

General Statements

15. *Primary prophylaxis*: In non-SLE patients with a high-risk aPL profile and concomitant thrombotic risk factors but no prior thrombosis, long-term thromboprophylaxis with low-dose aspirin is recommended (Ruiz-Irastorza et al. 2011).
16. *Secondary prophylaxis*: Patients with undetermined APS (arterial or thrombotic event and aPL) that do not fulfill laboratory criteria for APS should receive thromboprophylaxis in the same manner as APL-negative patients. Patients with definite APS and an unprovoked first venous event should receive anticoagulation for life with a target INR 2.0–3.0 (Ruiz-Irastorza et al. 2011).
17. *Caveats*: Some issues regarding the management of APS are yet to be determined. There is no consensus regarding the proper treatment for arterial events in definite APS. Should the patient receive anticoagulation with an INR target >3 or combination therapy with an anti-aggregant? Should the treatment of a low-risk APL-positive patient with a first provoked thrombosis be limited to 3–6 months? What is the treatment for patients in whom conventional anticoagulation is contraindicated? What is the best therapy for patients with refractory APS?
18. *Alternative treatments*: Yet to be determined are the roles of alternative treatments such as hydroxychloroquine, low molecular weight heparin, and statins especially in refractory cases (Branch 2011; Ruiz-Irastorza et al. 2011). Ongoing and future research may establish a role for rituximab, factor Xa inhibitors (dabigatran and rivoroxaban), or other novel agents for refractory thrombosis in APS patients (Scoble et al. 2011).

Conclusions

APS is an autoimmune disease characterized by recurrent thrombotic events and/or pregnancy morbidity in the presence of distinct autoantibodies. The risk for thrombotic events is dependent on the APL profile and includes the type, level, persistence, and number of antibodies involved; the presence of other risk factors; and the presence of SLE or other autoimmune diseases. The treatment of APS should be directed to subgroups based on clinical manifestations (thromboembolic events and/or pregnancy morbidity), immunological findings (which type of antibody, the titer and persistence, and the coexistence of triple positivity), and the presence of other autoimmune disease (primary or secondary APS).

Cross-References

- ▶ Antiphospholipid Syndrome, Clinical Manifestations
- ▶ Anti-phospholipid Antibody Mechanisms of Thrombosis
- ▶ Pregnancy in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Bramham K, Hunt BJ, Germain S, et al. Pregnancy outcome in different clinical phenotypes of antiphospholipid syndrome. Lupus. 2010;19:58–64.

Branch W on behalf of the Obstetric Task Force. Report of the obstetric APS task force: 13th international congress on antiphospholipid antibodies, 13th April 2010. Lupus 2011;20:158–64.

Cervera R, Font J, Khamashta M, et al. Antiphospholipid syndrome: clinical and immunological manifestations and patterns of disease expression in a cohort of 1000 patients. Arthritis Rheum. 2002;46:1019–27.
Cohen D, Berger SP, Steup-Beekman GM, Bloemenkamp KWM, Bajema IM. Diagnosis and management of the antiphospholipid syndrome. BMJ. 2010;340:1125–32.
Cosmi B, Legnani C, Tosetto A, PROLONG Investigators, et al. Usefulness of repeated D-dimer testing after stopping anticoagulation for a first episode of unprovoked avenous thromboembolism: the PROLONG II prospective study. Blood. 2010;115:481–8.
Danza A, Ruiz-Irastorza G, Khamashta M. Antiphospholipid syndrome in obstetrics. Best Pract Res Clin Obstetrics Gynecol. 2012;26:65–76.
Erkan D, Pierangeli SS. Antiphospholipid syndrome: Insights and Highlights from the 13th International Congress on Antiphospholipid Antibodies. Chapter 11: Ruiz-Irastorza G, Pengo V, Tektonidou MG, Ruiz-Arruza I, Cuadrado MJ, Khamashta MA. Task force report on the management of thrombosis in antiphospholipid syndrome. 3rd ed. New York: Springer; 2012. pp 167–179.
Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulant s are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. Blood. 2003;101:1827–32.
Heilman L, Schorsch M, Hahn T, Fareed J. Antiphospholipid syndrome and preeclampsia. Semin Thromb Hemost. 2011;37:141–5.
Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295–306.
Prandoni P, Prins MH, Lensing AW, AESOPUS Investigators, et al. Residual thrombosis on ultrasonography to guide the duration of anticoagulation in patients with deep vein thrombosis: a randomized trial. Ann Intern Med. 2009;50:577–85.
Ramires de Jesus GR, dos Santos FC, Oliveira CS, Mendes-Silva W, Ramires de Jesus N, Levy RA. Management of obstetric antiphospholipid syndrome. Curr Rheumatol Rep. 2012;14:79–86.
Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. Lancet. 2010;376:1498–509.
Ruiz-Irastorza G, Cuadrado MJ, Ruiz-Arruza I, Brey R, Crowther M, Derksen R, Erkan D, et al. Evidence-based recommendations for the prevention and long-term management of thrombosis in antiphospholipid antibody positive patients: Report of a task force at the 13th international congress on antiphospholipid antibodies. Lupus 2011;20:206–18.
Scoble T, Wijetilleka S, Khamashta M. Management of refractory anti-phospholipid syndrome. Autoimmun Rev. 2011;10:669–73.

Antiphospholipid Syndrome, Clinical Manifestations

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Synonyms

APS – clinical manifestations

Definition

Antiphospholipid syndrome is a systemic autoimmune disease characterized by various clinical manifestations (the most characteristic are thromboses and pregnancy morbidity) in the presence of elevated levels of antiphospholipid antibodies.

Antiphospholipid syndrome (APS) is a systemic disorder characterized by the presence of elevated levels of antiphospholipid antibodies (aPLs) and either thrombosis or pregnancy morbidity. As the latter 2 groups of clinical manifestations define the criteria for the diagnosis of APS, many other clinical manifestations are also prevalent in APS.

The major groups of clinical manifestations in APS are (Cervera et al. 2002, 2011a, b; Ramos-Casals et al. 2004; Rodrigues et al. 2010; Sherer and Shoenfeld 1998; Shoenfeld et al. 2008):

1. Thromboembolic manifestations
   (a) *Deep venous thrombosis and pulmonary emboli*: Thrombosis is one of the most common features of APS, and deep venous thrombosis (usually of the lower extremities) is the most common manifestation of thrombosis is APS. About one-third of the cases of deep venous thrombosis are associated with pulmonary emboli. Depending on their size, the pulmonary emboli may sufficiently impair

perfusion to reduce oxygenation. The clinical manifestations of pulmonary emboli include chest pain, dyspnea, tachypnea, decreased blood oxygen saturation, cardiac dysfunction, and sudden death.

(b) *Coronary artery disease* (*CAD*): Although this disease is found in many patients in the general population and is the leading cause of death in the Western world, the risk of CAD is even higher among patients with APS. Myocardial infarction occurs in about 7 % of APS patients, although some studies have reported a prevalence of up to 30 %. Objective evidence of coronary artery disease is found in 10 % of APS patients who also have SLE. In addition to aPLs, several risk factors contribute to cardiovascular diseases in these patients (smoking, hypertension, hypercholesterolemia, and diabetes mellitus), and therefore, interventions for these risk factors are of a great importance.

2. Recurrent miscarriage, pregnancy, and fetal morbidity
   (a) *Infertility*: The cause of infertility in some patients remains unexplained, and some investigators have suggested a possible involvement of aPLs. However, aPLs are normally associated only with recurrent miscarriages and obstetric complications.
   (b) *Recurrent miscarriage*: 2–5 % of women of reproductive age experience two or more miscarriages. Pregnancy loss during any stage of pregnancy may be the first and occasionally the only sign of APS. The characteristics of pregnancy losses vary in different research studies, but aPLs are generally associated both with early and late pregnancy loss. Most cases of miscarriage in women with and without APS occur during the early stages of pregnancy. Since APS is also associated with late pregnancy loss, the percent of women with APS and this type of miscarriage is relatively high. The presence of aPLs is important for the diagnosis, but it also has therapeutic and prognostic significance: A high rate of successful pregnancy can be anticipated while properly treated with anticoagulants. The subsequent miscarriage rate in women positive for aPLs is as high as 90 %, significantly higher than in women without aPLs.
   (c) *Pregnancy morbidity*: Preeclampsia is not a rare manifestation during pregnancy, but its frequency is higher among APS patients. In APS patients, preeclampsia usually occurs at an earlier stage of pregnancy and is more severe than in women without APS. Preterm delivery is also more frequent in women with APS (approximately 20 % of cases) and is usually the result of childbirth induced by the physician due to preeclampsia and fetal growth retardation. This latter complication of APS is usually the result of placental dysfunction, and it has been reported in 10–30 % of pregnancies in women with APS.
3. Heart and respiratory manifestations
   (a) aPLs have an important role in heart valve damage both in primary APS and in lupus; they are found in 90 % of lupus patients who have heart valve injury and in only 40 % of lupus patients without heart valve disease. The most frequent manifestation of heart valve disease, both in APS and in lupus (SLE), is heart valve thickening. The valve most frequently involved is the mitral valve, followed by the aortic valve. Thickened heart valves have impaired function. About one-quarter of patients with APS and lupus have mitral valve disease, which results in mitral regurgitation. This disease is significant in 5 % of the patients and results in heart failure. Another relatively common manifestation of valve disease in APS is the development of heart valve vegetations. Another interesting manifestation in APS is pseudo-infective endocarditis, which imitates the clinical manifestation of infective endocarditis with signs and symptoms of fever, heart valve vegetations, heart murmur, and

splinter hemorrhages. However, no infective agent can be detected by blood cultures, but moderate to high titers of aPLs can be found (similar to "marantic endocarditis").

(b) *Cardiac thrombosis*: APS is characterized by hypercoagulability, which can also manifest as a thrombus within the cardiac chambers. A thrombus in this location can cause severe heart dysfunction as well as producing emboli that could cause occlusion of vessels that supply the brain, kidneys, and other vital organs.

(c) *Pulmonary arterial thrombosis*: This is a rare manifestation of APS. However, as thrombosis can occur in every vessel in the human body, it can also occur in one of the major arteries of the lung. Thrombosis can also affect smaller lung vessels.

(d) *Pulmonary hypertension*: Pulmonary hypertension occurs in 2–3 % of APS patients and is usually the result of recurrent pulmonary emboli.

(e) *Pulmonary hemorrhage*: Pulmonary hemorrhage is characterized by cough, fever, dyspnea, and hemoptysis.

4. Skin involvement in APS

(a) *Livedo reticularis*: This rash is characterized by a netlike reddish-blue discoloration beneath the skin. Occasionally, similar phenomenon can be found in healthy subjects following cold exposure. Livedo reticularis can be found in about one-fourth of APS patients, more frequently in women than in men, and more frequently in patients having secondary APS associated with lupus.

(b) *Skin ulcers*: These occur in 55 % of APS patients. These usually manifest as small and painful lesions with a diameter of 0.5–3 cm, with a round- or star-shaped border circled by brown-purple border and intradermal hemorrhage.

(c) *Digital necrosis*: This occurs in approximately 3 % of patients with APS. The risk factors for digital necrosis in APS are smoking, hypertension, and oral contraceptive use.

5. Central nervous system and APS

(a) *Stroke*: Strokes in APS can be the result of intracerebral thrombosis, thrombosis in the large vessels supplying the brain (the carotid arteries in the neck or the vertebral arteries in the spinal column), or due to emboli of thromboses originating in the aorta or heart. The presence of aPLs is associated with a 2–7 times increased risk for stroke compared with patients without aPLs. Strokes in individuals with APS also occur earlier, several decades prior to their occurrence in the general population.

(b) *Venous sinus thrombosis*: Thromboses of the venous sinuses can be severe and lead to brain infarcts.

(c) *Sneddon's syndrome*: This is defined as recurrent strokes and livedo reticularis. The prevalence of aPLs in Sneddon's syndrome is 50–85 %.

(d) *Epilepsy*: The presence of aPLs is associated with epilepsy in patients with lupus. A possible pathogenic mechanism of epilepsy in APS is thrombosis followed by brain infarct and scar formation. aPLs could also promote epilepsy by having a direct effect on the brain, such as decreasing the activity of the neurotransmitter GABA.

6. Hematological manifestation of APS

(a) *Thrombocytopenia*: Thrombocytopenia occurs in 20–40 % of individuals with APS, but usually in a mild form with platelet counts above 50,000 per mL. Severe thrombocytopenia is found in the minority (5–10 %) of patients. Severe bleeding rarely accompanies the low platelet counts.

(b) *Anemia*: In some cases of autoimmune hemolytic anemia, aPLs (usually anticardiolipin antibodies) can be detected. A minority of patients have both thrombocytopenia and anemia (Evan's syndrome), which can be detected in about 5 % of lupus and 10 % of the patients with primary APS.

7. Other clinical manifestations in APS

(a) *Renal injury*: The kidneys are a target affected in many systemic diseases such

as diabetes mellitus, hypertension, and SLE. In APS, renal artery thrombosis can also occur, with arterial stenosis or emboli blocking the renal artery. Disruption of renal blood flow for any reason leads to hypertension and, occasionally, to severe and life-threatening hypertension. Renal vein thrombosis can also be part of APS; these thrombi occur mainly in the presence of nephrotic syndrome. Thrombosis can also occur within the small vessels of the kidneys, including arterioles, venules, and even in the glomerular vessels.

(b) *Intestinal manifestations*: Thrombosis of the intestinal arteries can result in intestinal necrosis. An early manifestation of intestinal thrombosis is abdominal angina resulting from narrowing and incomplete occlusion of the intestinal arteries.

(c) *Budd-Chiari syndrome*: In APS, Budd-Chiari syndrome is caused by interference of hepatic venous drainage. Liver venous thrombosis can be accompanied by thrombosis of larger veins such as the inferior vena cava. Budd-Chiari syndrome can be acute or chronic, can completely resolve, or can lead to death due to hepatic failure. aPLs have been found in many patients with liver cirrhosis, portal vein hypertension, and hepatitis C virus infection.

(d) *Hearing impairment in APS*: Hearing loss can result from impairment of the blood supply to the cochlear nerve. aPLs have been detected in patients with sudden hearing loss, with different studies reporting frequencies varying from very low to approximately one-fourth of these patients.

(e) *APS and the eyes*: Central nervous system involvement may affect vision in both eyes. However, single eye involvement also occurs in APS, and the underlying mechanism is decreased blood supply to the eye or an inflammatory reaction specific to that eye. Another pattern of visual injury is the result of injury to the cranial nerves that supply eyeball muscles or injury to the optic nerve itself. The symptoms presented by patients having ocular manifestations of APS are diverse and can include double vision, ocular pain, headache, visual fields impairment, and transient visual loss is one or both eyes.

(f) *Bones and joints in APS*: Bones, muscles, and joints are not usually affected in patients having APS. However, as APS is frequently secondary to other autoimmune diseases, including lupus, these primary diseases can have joint manifestations, such as arthritis. Whereas primary APS is not characterized by arthritis, arthralgias are relatively common in APS. In addition, bone marrow necrosis has also been reported in some patients with APS. The main injury to the bones in APS is avascular necrosis, which results from blood supply impairment.

In conclusion, APS is a multisystem autoimmune disease that is characterized mainly by thrombosis and pregnancy morbidity. However, many other clinical manifestations are common among patients with APS, and therefore clinical suspicion of APS should be high in the presence of typical clinical presentations, especially among patients with other autoimmune diseases.

Cross-References

- ▶ Anti-phospholipid Antibody Mechanisms of Thrombosis
- ▶ Antiphospholipid Syndrome Treatment

References

Cervera R, Piette JC, Font J, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum. 2002;46:1019–27.

Cervera R, Tektonidou MG, Espinosa G, et al. Task force on catastrophic antiphospholipid syndrome (APS) and non-criteria APS manifestations (II): thrombocytopenia and skin manifestations. Lupus. 2011a;20:174–81.

Cervera R, Tektonidou MG, Espinosa G, et al. Task force on catastrophic antiphospholipid syndrome (APS) and non-criteria APS manifestations (I): catastrophic APS, APS nephropathy and heart valve lesions. Lupus. 2011b;20:165–73.

Ramos-Casals M, Cervera R, Lagrutta M, et al. Clinical features related to antiphospholipid syndrome in patients with chronic viral infections (hepatitis C virus/HIV infection): description of 82 cases. Clin Infect Dis. 2004;38:1009–16.
Rodrigues CE, Carvalho JF, Shoenfeld Y. Neurological manifestations of antiphospholipid syndrome. Eur J Clin Invest. 2010;40:350–9.
Sherer Y, Shoenfeld Y. Anti-phospholipid autoantibodies-do they have a pathogenic role in infertility? Scand J Rheumatol. 1998;107:40–3.
Shoenfeld Y, Twig G, Katz U, Sherer Y. Autoantibody explosion in antiphospholipid syndrome. J Autoimmun. 2008;30:74–83.

Atherosclerosis and Cytokines

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Synonyms

Atherosclerosis – Arteriosclerosis; atheroma establishment; blood vessel narrowing; vessel wall hardening; ischemic heart disease; coronary artery disease; inflammatory disease

Inflammation – *Dolor*; *rubor*; *calor*; *tumor*; *functio laesa*; pain; redness; temperature; swelling; impaired function; cell recruitment; cytokine production; secondary mediators; secondary functions; cytokine-mediated

Cytokines – Hormone-like mediators; growth factors; proinflammatory cytokines; anti-inflammatory cytokines; monokines; lymphokines; intercellular communication mediators; LMW (low molecular weight) proteins; immune cell-released; inflammatory cell-released; stimulated proteins; primary inflammatory mediators

Definition

Atherosclerosis is a life-threatening thickening of the vessel wall, resulting in a reduction or failure of oxygen nourishment of heart or brain tissues. It is the leading cause of infarction and stroke in the industrial countries. The development of atherosclerosis, a process termed atherogenesis, is crucially controlled by inflammatory processes. Cytokines are central regulators of inflammation, thus, they are essential for atherogenesis. The role of cytokines for atherogenesis has been indicated by measurement of cytokines in blood samples and by in vitro cell-culture experiments and has finally been proven using cytokine-gene-deficient animals.

Historical Background

It is probably not possible to determine unequivocally the first mentioning of atherosclerosis in ancient history, may it be of Asian or European origin. More recently, it has been Leonardo da Vinci in the sixteenth century who investigated the anatomy of vessels and discussed the phenomenon of vessel narrowing. The terms arteriosclerosis and atherosclerosis have then been introduced in the nineteenth century. Already at this time, inflammation was linked to atherosclerosis by a number of scientists including Joseph Hodgson and Rudolf Virchow. However, it took more than a century that this idea was seized again and investigated in more detail. Due to the discovery of the inflammatory mediators in the twentieth century, the contribution of inflammation (i.e., its mediators) to atherogenesis is now well established (for further historical reading see Nieto (2012)).

Atherosclerosis

Cardiovascular diseases are a major cause of morbidity and mortality in the industrial countries. Atherosclerotic alterations in the vessel wall are the underlying cause of many of these diseases. Atherosclerosis has been associated clinically with risk factors such as hypercholesteremia, diabetes, diet, obesity, metabolic syndrome, hypertension, smoking, and shortcomings in physical activity, gender

(man > woman), and age (older > younger). On the other hand, it is now well accepted that inflammatory pathways are involved in the development and progression of atherosclerosis (Hansson and Libby 2006; Ross 1999). Exogenous or endogenous compounds or conditions, representing the risk factors, can be determined, and cells of the immune system or the vessel wall, by responding to these challenges, may initiate the inflammatory response in or on the vessel wall (Loppnow et al. 2011). The exogenous activation pathways may include bacterial components, such as endotoxin (LPS, lipopolysaccharide) or other bacterial products. The endogenous activators of inflammation may include dietary or metabolic products, stress, or even inflammatory mediators, such as cytokines themselves. Activation of inflammation results in leucocyte invasion into the vessel wall, as well as migration and proliferation of local smooth muscle cells. These cellular processes are influenced by fluctuations in cytokine production and reactivity, by altered responses to blood flow, or by the "inflammatory burden" caused by the exogenous or endogenous triggers, representing the risk factors. The putative steps in atherogenesis (compare Libby 2012) are summarized in Table 1.

Atherosclerosis and Cytokines, Table 1 Inflammatory steps in atherosclerosis[a]

Activation of adhesion molecules on the endothelium (presumably by exogenous or endogenous inflammatory triggers related to the risk factors, which activate cytokine production)
Adhesion and penetration of monocytes and, subsequently, other cells, into the intima and activation of the intravascular inflammation process by cytokines
Accumulation and further attraction of cells by chemokines
Transformation of monocytes to foam cells in response to cytokines and cholesterol uptake
Migration, proliferation, as well as death, of vascular smooth muscle cells, in response to invading-cell-derived cytokines or autocrine cytokines
Alteration of plaque stability by cytokine-mediated enzyme production
Modification of the fibrous cap and, in the worst case, rupture of the plaque
Thrombosis, resulting in stroke or myocardial infarction

[a]Modified after Libby (2012)

In early atherosclerosis, innate mechanisms, such as enhanced cytokine (i.e., IL-1 (interleukin-1) or TNF (tumor necrosis factor)) production, as well as monocyte or granulocyte invasion, may lead to endothelial dysfunction, enhanced coagulation, or enhanced plasma protein leakage. Enhanced adhesion to the endothelium or increased numbers of "patrolling" monocytes may increase accumulation of leukocytes in the vessel wall and promote the local inflammatory response. The later phases of plaque development are characterized by an immense accumulation of various cell types in the vessel wall, as well as by accumulation of extracellular matrix compounds and cholesterol. In the wall, monocytes eventually become foam cells, which form a core region in the plaque. Besides these cells, which represent the numerical largest part of the invading cells, T cells and other cells have been detected in the plaque. Among the cells involved in atherogenesis are also mast cells. They may contribute to plaque vulnerability in late atherosclerosis by production of cytokines and, subsequently, by production of enzymes, such as matrix metalloproteases, cathepsins, chymases, or other proatherogenic proteases. These processes may reach a time point where they become irreversible and vessel wall architecture is destroyed. The ratio of migrating and emigrating cells appears to be important for progression of plaque development. The vulnerability of the plaque in the late phase of atherosclerosis is a very critical element in the fatal outcome of atherosclerosis in many cases. Inflammation and the resulting activation of proteolytic enzymes has been suggested to be an important determinant of plaque stability.

Thus, formerly regarded as a lipid disease, more recently, inflammation is supposed to be an important factor in atherogenesis. Based on the "response to injury" proposal by Russel Ross (Ross 1999), vascular inflammation may be initiated by one or more of various pathways (including activation by lipids). Extending this suggestion, the risk factors, previously determined in clinical research, may be epitomized

by biochemical or cell-biological compounds or functions, which regulate the extent of inflammation in the vessel wall. Although the past decades have taught researchers immensely about the biology of atherosclerosis, many important questions remain open. Among them is the question of the location of the atherosclerotic lesion, that is, why are particular types of vessels and particular regions in these vessels preferentially haunted by atherosclerosis? Another question is the importance of the sequence and intensity of the activation of inflammation in the early steps of atherogenesis by the various "risk factor-related" compounds or situations. Also of importance is the question at what time the "point of no return" in the lesion is trespassed, where cellular repair mechanisms keeping inflammation in check are no longer effective.

Inflammation

The term inflammation is derived from the Latin *inflammo, inflammatio* (i.e., to enkindle, to inflame; inflammation). The phenotype of inflammation has been described since the first and second century AD (Celsus; Galenus) by pain, redness, temperature, swelling, impaired function (i.e., *dolor*, *rubor*, *calor*, *tumor*, and *functio laesa*). These symptoms are caused by a variety of assaults, including infections, injury, or cell damage. Inflammation is a more generalized, that is, less specific, response of the body, in order to initiate repair or defense mechanisms. Thus, it is related to the innate immune pathways. Although inflammation is essential for healing processes, a dysregulated, in particular an enhanced inflammation, may cause chronic inflammatory diseases. In the inflammatory process, many different systems of the body are involved. Thus, prostaglandins, leukotrienes, histamines, bradykinins, complement factors, as well as coagulation and fibrinolysis factors play a role in inflammation. In this inflammatory reaction of the body, the cytokines have a central regulatory role. The cytokines cause the production and/or regulation of the above mediators, that is, many of these mediators are secondary to the cytokines. In turn, some of the mentioned substances may interfere with cytokine production. Since the cytokines have such a central and complex role in inflammation, and inflammation is so importantly involved in atherosclerosis, this overview concentrates on the cytokines and their relationship to atherosclerosis.

Usually, an inflammatory response is induced upon an insult, kept active until the insult is managed, and downregulated as soon as it is no longer required. This is due in part to the short half-life of the involved mediators. On the other hand, there are regulatory processes at various levels, which keep the inflammation in check. These regulatory levels are discussed below in the cytokine paragraphs. If the cause of the insult cannot be resolved or the inflammatory processes are dysregulated, chronic inflammatory diseases may develop.

The insults causing the inflammatory response do that by activating a cellular response. The cells involved in this response are, in the first line, monocytes and granulocytes. They are the basis of the so-called innate immune response in inflammation. Only later, their response will attract and activate cells of the adaptive immune system, which react much more specific to the insult(s). In the innate immune response, the cells are activated by the compounds causing the insult via pattern-recognition receptors (PRR). These receptors do not react very specifically to particular activators, as it is the case in the adaptive immune system, which is antigen specific. The PRRs can be activated by a variety of activators, although these activators must be of a comparable structure, type, or "pattern." These receptors react with substances containing a so-called "pathogen-associated molecular pattern" (PAMP) or a "damage/danger-associated molecular pattern" (DAMP). A variety of such receptors has been identified (e.g., TLR (*toll*-like receptor), NLR (NOD (nucleotide-binding oligomerization domain containing)-like receptor), CLR (C-type lectin receptor), etc.) and their activators described (compare (Loppnow et al. 2011)). Due to the broad specificity of these receptors and their

location in high concentrations on monocytes and granulocytes, the PAMPs or DAMPs quickly and potently activate the cytokine production and the production of subsequent inflammatory compounds.

Cytokines

The term cytokine is derived from the Greek *kýtos* (vessel, jar, cell) and *kinein* (movement), meaning as much as "interacting between cells." These hormone-like mediators regulate a variety of important cell functions, including cell differentiation, tissue repair, immune response, inflammation, contraction, or cell death. More than 150 different cytokines have been described in the past. In the beginning, cytokine terminology has been related to the function of the cytokine (e.g., "endogenous pyrogen" causes fever, it is now termed interleukin-1 (IL-1); "T cell growth factor" stimulates T cell proliferation, it is now termed IL-2). However, some cytokines have been discovered in parallel in different assay systems, because each cytokine may exert different functions. For example, IL-1 was isolated as the inducer of fever. In this situation, it was called endogenous pyrogen (EP). In addition, IL-1 was also isolated as the protein inducing the stimulation of IL-2 production of T cells and was termed lymphocyte-activating factor (LAF). Later, upon isolation of the respective cDNAs, it turned out that these and many other proteins, described for other functions, are identical and, finally, were called IL-1. Just to give one example, others exist for other cytokines.

Also, other previous specifications have taken the cellular source into account. The first interleukin, that is, IL-1, was identified originally in monocytes, thus, the terminus "monokine" was coined for such monocyte-derived cytokines. On the other hand, IL-2 was described in lymphocytes and, therefore, called a "lymphokine." This turned out to be not practical, since it became clear afterwards that many different cells, not only monocytes or lymphocytes, but also tissue cells, can produce a particular cytokine.

On the other hand, the designation pro- and anti-inflammatory has been used to show the function of cytokines in reactions of the body. This brings together many different types of cytokines, however, is not applicable to all cytokines, since some cytokines are not primarily involved in inflammation. Still, this terminology is useful, since it refers to biological or medical functions.

Since the discovery of the first cytokines, a variety of cytokine groups have been determined. Starting from the functional description, the molecular characteristics and the relation to the corresponding receptors have been integrated in the establishment of these groups (summarized in (Loppnow 2001)). Although some editors have put some efforts into a comprehensive description of the cytokine groups (Thomson and Lotze 2003), no current handbooks or summary on this topic are available. This is probably due to the myriad of information available regarding the cytokine topic. Indeed, at the day of writing this text, a PubMed search with the keyword "cytokine" resulted in 580,265 titles. The cytokine groups include the interferons (IFN), the interleukins (IL), the tumor necrosis factors (TNF), the colony-stimulating factors (CSF), the chemokines, various growth factors, and the virokines. The virokines, as well as the viroceptors, are cytokine-related products, such as IL-10 homlogous or caspase-1 inhibiting proteins, as well as IL-1 receptor-like or TNF receptor-like proteins, respectively. The microorganisms, in particular viruses, may thwart the immune response against themselves by causing the infected cell to produce these substances, thereby reducing inflammation. The original activities, as well as additional activities of the respective cytokine groups, are mentioned in a very general way in Table 2. This table does not make any claim of being complete; rather, it shall enunciate the capacity of given cytokines, or cytokine groups, to fulfill various functions in parallel, functions which may be very different from each other. This – pleiotropy – is a central feature of cytokines, always to be considered in research involving cytokines. The table also

Atherosclerosis and Cytokines, Table 2 Cytokine groups

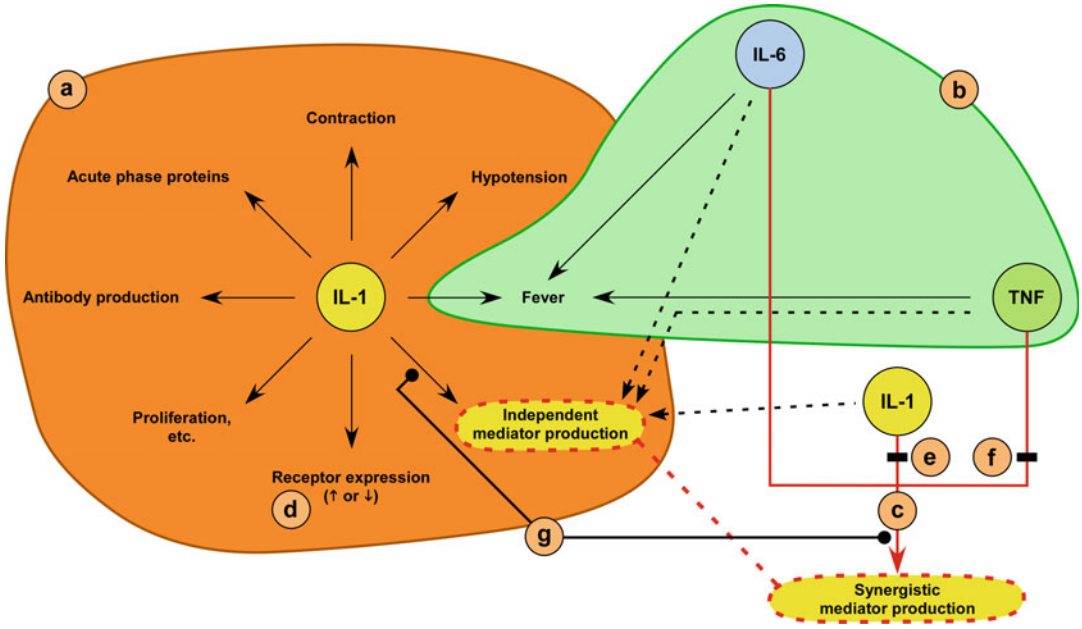
Group	Original description	Additional functions
Interferon	Antiviral	Immunoregulation
		Macrophage activation
		MHC-class I induction
Interleukin	Immunoregulation	Regulation of inflammation
		Regulation of other mediators
		Regulation of enzyme production
		Chemotaxis
Tumor necrosis factor	Tumor necrosis	Immunoregulation
		Regulation of apoptosis
Colony-stimulating factor	Hematopoiesis (i.e., cell differentiation of precursor cells)	Immunoregulation
Chemokine	Chemotaxis	Immunoregulation
		Adhesion
Growth factor	Proliferation	Immunoregulation
Virokine/viroceptor	Cytokine/cytokine receptor mimetic	

Abbreviations: *MHC* major histocompatibility complex

gives an allusion to another central feature of cytokines, the – redundancy – of the cytokine system. Redundancy means that various different cytokines, or cytokine groups, may have the same function, for example, interleukins, TNFs, and CSFs have immunoregulatory functions (compare also Fig. 1).

Many of the cytokines have been characterized at the molecular level. As it turned out, defined molecular characteristics do exist. Although the classification of the various cytokine groups with respect to their 3-dimensional structure is not completely uniform in the literature, some well-defined groups have been suggested (Nicola 1994). These groups are differentiated basically by protein-biochemical criteria. These groups and some examples are summarized in Table 3. One group is characterized by the presence of a set of beta sheets (long chain β-sheet cytokines). Some contain four (long or short) alpha helices (4-α-helical bundle cytokines) in their structure, and two other groups contain various elements (short-chain α-β-cytokines; mosaic cytokines).

The functions of cytokines are mediated by binding of the cytokines to the respective receptor. As in the case of the cytokines, the receptors also share some particular structural characteristics. There are three groups of cytokine receptors (in the broader sense of the word): (1) the cytokine receptor group in the narrow sense of the word, including four families (C1–C4 in Table 3); (2) the receptor kinase group with several subfamilies (K in Table 3); and (3) the chemokine receptor group (7-M in Table 3). (1) The receptors of the cytokine receptor group in the narrow sense are classified by their extracellular molecular characteristics. This group contains four families. The first is determined the "cytokine receptor family I" or "hemopoietin receptor family" (C1 in Table 3). The "cytokine receptor family II" or "interferon receptor family" (C2 in Table 3) utilizes similar signaling pathways, although it has different extracellular characteristics. The third family (C3) is the "TNF/NGF receptor family," which is connected to caspase or NF-kB (nuclear factor "kappa-light-chain-enhancer" in B cells) signaling. The "immunoglobulin receptor family," including the IL-1 receptor, also utilizes NF-kB, as well as MAP (mitogen-activated protein) kinase pathways. (2) The "kinase receptor groups" (K in Table 3) are classified by their intracellular kinase domains. They contain various types of tyrosine or serine/threonine kinases. The signaling pathways are in general different from those of the cytokine receptor group; they include Ras (rat sarcoma)/Raf



Atherosclerosis and Cytokines, Fig. 1 *Cytokine regulatory and interaction pathways:* The different parts (**a**-**g**) of the figure explain by representative examples the particular characteristics and/or pathways schematically. (**a**) **Pleiotropy**: one cytokine has a number of different functions; in this part of the figure (orange area), some IL-1 functions are mentioned; many other functions are not included but only referred to by the "*etc*." in "proliferation, *etc*." (**b**) **Redundancy**: one function can be induced by more than one cytokine; the green area of the figure indicates that IL-1, IL-6, and TNF can induce fever and replace each other inducing this symptom. (**c**) **Synergism**: here, mediator production is used as an example; the *red lines* and *red arrow* indicate that the mediator production in the simultaneous presence of IL-1, IL-6, and TNF is synergistic. The broken red line and the yellow fields surrounded by the broken red frame indicate the comparison of the "mediator production" by each of the three cytokines independently (broken black lines; black arrow), as compared to the production following parallel stimulation (red lines; red arrow). A synergism is present if the "synergistic mediator production" is much higher (overadditive) than the sum of the three "independent mediator productions" (e.g., 5,000 % vs. 100 + 100 + 100 %). (**d**) **Receptor expression**: cytokines can only cause a function if their respective receptor is expressed. Cytokines themselves can regulate the expression of their receptors. Thus, they may cause the expression (upregulation) or the disappearance (downregulation) of their own or other cytokine receptors, thereby making the cells responsive or irresponsive to themselves or the other cytokine, respectively. As an example, IL-1 may downregulate its own receptor, whereas other cytokines, such as IL-4, may upregulate the IL-1 receptor. (**e**) **Competitive inhibitors**: if a protein can bind to the receptor, without causing a signal, thereby preventing the binding of the substrate, competitive inhibition may take place. In the cytokine universe, the IL-1 receptor antagonist and the IL-36 receptor antagonist, also an IL-1 family member, are examples. They circumvent binding of the IL-1 or IL-36, respectively, thereby preventing activation of the cell. (**f**) **Soluble receptors**: the extracellular portion of a receptor may be released from the cell by enzymatic digestion. In some cases, this soluble form of the receptor (e.g., IL-1-, IL-6-, or TNF-soluble receptor) can still bind the substrate; this prevents the respective cytokine from binding to membrane-associated receptors, thereby preventing the activation of the cell. (**g**) **Anti-inflammatory cytokines**: particular cytokines can reduce the production or function of other cytokines. As an example, IL-10 may downregulate cytokine production by TH1 cells or monocytes. Such an inhibition is indicated in the figure by the *round-headed arrows*

(rapidly growing fibrosarcoma), phospholipase, Smad (Sma- and MAD-related proteins), or TAK (transforming growth factor-β–activated kinase) pathways, to mention only a few. (3) The chemokine receptors are particular, since they are seven-membrane-spanning-G-protein-coupled receptors, like the thrombin or the endothelin receptor.

In its schematic overview, Table 3 also points out that there is a functional relationship between

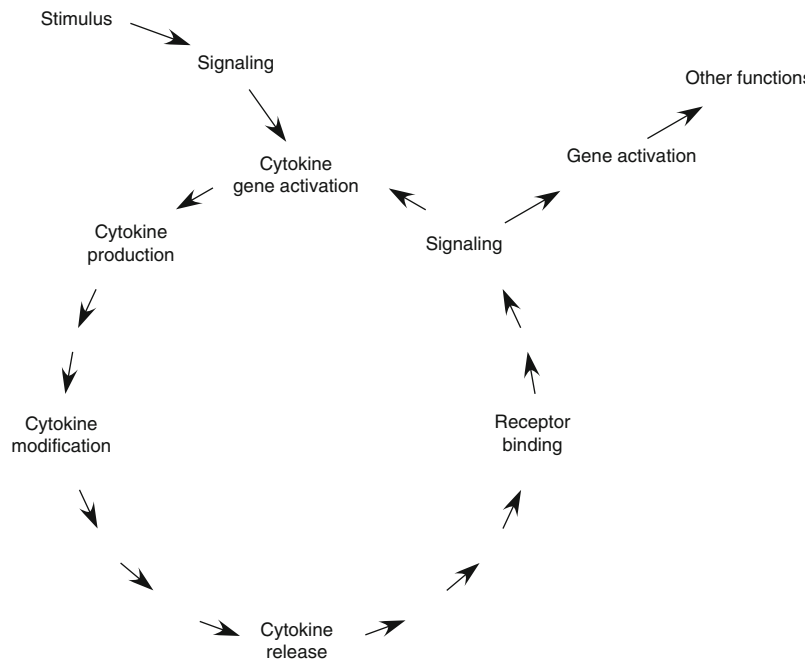
Atherosclerosis and Cytokines, Table 3 Cytokine groups and their relation to the respective cytokine receptor groups

Group	Family	Structural characteristic	Examples	Related receptor families[a]
Long chain β-sheet cytokines	IL-1 family	β-trefoil	IL-1[b]	C4
			FGF	K
	Cystein-knot family	-s-s-groups; β-sheets	PDGF, TGF-β, NGF	K
			LT	C3
	TNF family	β-jelly roll	TNF	C3
4-α-Helical cytokines	Short chain	< 25 aa / helix	IL-2, IL-3, IL-4	C1
			IFN-γ	C2
			M-CSF	K
	Long chain	> 25 aa / helix	IL-6, IL-11, G-CSF	C1
			IL-10, IFN-β	C2
Short chain α-β-cytokines		-s-s-groups; β-sheets	EGF, TGF-α, IGF	K
			IL-8, MCP, Rantes	7-M
Mosaic cytokines		Various	HRG, HGF	K

[a]C1–C4 are the four families of the cytokine receptor group; K are receptors of the receptor kinase group (yellow), 7-M is the chemokine receptor group (orange), Modified after Nicola (1994)
[b]Abbreviations: *CSF* colony-stimulating factor, *EGF* epidermal growth factor, *FGF* fibroblast growth factor, *HGF* hepatocyte growth factor, *HRG* heregulin, *IFN* interferon, *IGF* insulin-like growth factor, *IL* interleukin, *LT* lymphotoxin, *MCP* monocyte chemoattractant protein, *NGF* nerve growth factor, *PDGF* platelet-derived growth factor, Rantes regulated on activation, normal T expressed and secreted, *TGF* transforming growth factor, *TNF* tumor necrosis factor

the cytokines and their respective receptors: cytokines more prominently related to immunoregulation or inflammation are preferentially substrates of the receptors of the cytokine receptor group (C1–C4), whereas growth factors bind preferentially to receptor kinase family receptors (marked in yellow). The chemokines are interacting with the seven membrane spanning chemokine receptors (marked in orange). Thus, finally it turns out that the cytokine function is related (one even may say caused) by the subsequent signal-transduction mechanisms. However, for reasons of space limitation, this topic is not discussed here in more detail (for more information compare Fig. 2 in (Loppnow 2001)).

For the comprehension of the role of cytokines in pathogenesis, knowledge of some particularities of cytokines and their pathways is important. Two important characteristics of the cytokines have already been mentioned above. These were the pleiotropy and the redundancy in the cytokine system. Simply spoken, pleiotropy means that most cytokines do not only exert one particular function but can activate a variety of functions. IL-1 is a good example for a pleiotropic cytokine, since it is involved in a great number of biological functions (Fig. 1a, orange area) important for pathogenesis of many diseases (Dinarello 1996). In Fig. 1, redundancy (b) is depicted by the capacity of three different cytokines to induce fever (green area), that is, one particular function can be activated by different cytokines. This is a problem, if a therapy is based on blocking of a cytokine, since a failure of blocking may appear, due to the capacity of another cytokine to perform that particular function also, thereby replacing the original one. In the body, not only one cytokine will be present. Thus, the cytokines may interact in a synergistic fashion, that is, the effect induced by (c) three cytokines (red lines) in combination may be much higher (overadditive, synergistic) than the separate effects induced by the single cytokines (dashed black lines) summed up (indicated by yellow areas with red dashed lines). Another important regulatory pathway of cytokines is that they can regulate the receptor expression (d) of their own or other cytokine receptors. Upregulation of a certain cytokine receptor will make a cell responsive for a cytokine, whereas downregulation will make the cell irresponsive. This means that not only the concentration of a given cytokine is important



Atherosclerosis and Cytokines, Fig. 2 *Stimulation, production, and response to cytokines.* Activation of cytokine production, the subsequent modification and release pathways, and the following binding to receptors may lead to further production of atherosclerosis-related cytokines or to other functions relevant for atherogenesis. At many positions in this cascade of reactions, approaches for therapeutic attempts appear possible.

for its activity, but also the presence of a functioning receptor, indicating the complex regulation pathways involved in regulation of cytokine function. Other negative regulators of cytokine functions (in addition to receptor downregulation) are competitive inhibitors, such as receptor antagonists (e) which are described for some IL-1 family members. Furthermore, soluble extracellular portions of the respective receptors (f), for example, the soluble TNF receptor (soluble TNF R), which may bind and, thereby, inactivate the particular cytokine, are also proposed negative regulators. Finally, anti-inflammatory cytokines (g) may either block the cytokine production or the function of certain cytokines in various ways.

The above paragraph indicates the complex interactions of cytokines. However, these interactions are only a part of the cytokine regulatory system. In this paragraph, an attempt is made to bring the different levels of regulation together in an overview (Fig. 2). Cytokines are in general produced after stimulation. In the case of atherogenesis, it may be assumed that risk factor-related compounds, such as cholesterol, stress, infectious agents, or cytokines themselves, may activate cells of the blood or the vessel wall. They will do so by binding to the respective receptors, initiating intracellular signaling, which finally activates cytokine genes. In the activation of the production of some cytokines, for example, of IL-1, NF-kB plays a central role. Upon cytokine gene activation, the respective cytokine is produced in the cell. Some cytokines are modified then, for example, by glycosylation (e.g., IL-6 or IFN); others are produced as precursors (e.g., IL-1) and require further enzymatic processing in order to become active. In the case of IL-1β, this "processing" is regulated by activation of caspase-1, the enzyme responsible for IL-1β maturation. This step is regulated by a multiprotein complex termed "inflammasome," which has a central position in many inflammatory processes (Tschopp and Schroder 2010). Thus, IL-1β can only mature if both, the genes of itself and caspase-1 are active. Most cytokines contain a signal peptide, which makes them applicable for the classical release through the endoplasmic reticulum – Golgi release pathway. In case of IL-1, other pathways, such as vesicles, membrane transporters, or cell lysis, are proposed (Eder 2009). Upon release of the cytokine, it may bind to its specific receptor in an autocrine, paracrine, or endocrine fashion,

Atherosclerosis and Cytokines, Table 4 Examples of cytokines linked to atherogenesis[a]

Cytokine	Inflammatory		Producers	Putative functions in atherosclerosis
	Pro	Anti		
Interleukin-1	+		M[b] S E T	Potent EC and SMC activator; activates SMC migration and proliferation; activates enzyme production; increase fatty streak formation; decrease lesion size upon knockout; alter cholesterol and HDL levels; alter cell composition of the plaque
Interleukin-6	+	+	M S E	Activates SMC proliferation; activates enzyme production; activates acute phase protein production; alter lesion size; biphasic effects discussed
Interleukin-10		+	M T B	Anti-inflammatory activities; increase lesion formation upon knockout; risk factor
Interleukin-18	+		M S E	Proinflammatory activities; alter lesion size; T cell differentiation
Tumor necrosis factor	+		M S	Causes SMC apoptosis; regulates scavenger receptors; cell activator; decrease lesion size upon knockout; risk factor
Fractalkine	+		M S E	Monocyte attraction
M-CSF	+		M S E	Cell activator; chemotaxis

[a]This table summarizes a few cytokines suggested to be involved in atherogenesis. A more complete summary can be found in the excellent work of (Tedgui and Mallat 2006) and (Kleemann et al. 2008)
[b]Abbreviations: *B* B cells, *E* endothelial cells, *M* monocytes/macrophages, *M-CSF* macrophage-colony-stimulating factor, *S* smooth muscle cells, *T* T cells

meaning, it binds to the same cell, to another cell, or to a cell at another location. This activates intracellular signaling, which on the one hand may keep the cytokine gene activation active for another cycle of production. However, on the other hand, other genes may also be activated, resulting in other inflammatory functions. Many of these regulatory steps appear to be candidates for therapeutics targeting reduction of inflammation.

Evidence for the Role of Cytokines in Atherosclerosis

The role of cytokines in inflammation is apparent from the above paragraphs. What then indicates that cytokines are indeed involved in atherosclerosis? Research has focused at various levels of investigation in order to answer this question: (1) if cytokines are important for cardiovascular diseases, they may be present in the plasma of patients. Thus, a great number of clinical studies have been performed. Cytokines have been found in patients suffering from cardiovascular diseases, and some treatments have altered the cytokine levels (compare (Loppnow et al. 2008; Tedgui and Mallat 2006) for further reading). (2) A role of cytokines in cardiovascular diseases, such as atherosclerosis, would be further supported if the cells involved in plaque development, such as monocytes, granulocytes, endothelial cells, and smooth muscle cells, would be able to produce cytokines and interact by means of these mediators. This has been shown in an overwhelming wealth of publications. These data indicate that the cited cells can produce inflammatory mediators, such as IL-6 or IL-1, can respond to these substances, and can interact by cytokines (Chen et al. 2009; Loppnow and Libby 1990; Loppnow et al. 1998; Loppnow et al. 2008). (3) Since the patient and the in vitro cell-culture data indicated the importance of cytokines, efforts to prove the importance directly were performed in animals. Mice containing genetic deficiencies of the LDL (low-density lipoprotein) receptor or ApoE (apolipoprotein E) develop atherosclerosis upon cholesterol-rich chows. Crossing such animals with animals lacking cytokine genes (e.g., lacking the IL-1 receptor gene or the IL-1β gene) resulted in reduced atherosclerosis (Kleemann et al. 2008; Chi et al. 2004; Kirii et al. 2003). These data provided final evidence for the essential role of

cytokines in atherosclerosis. Cytokine research in this field has now reached a point where cytokine compounds are used in patients in clinical trials (Ridker et al. 2011).

Conclusion

There is now undisputed evidence that cytokines, the most prominent mediators of inflammation, are importantly involved in development of atherosclerosis (compare Table 4). The many regulatory steps in the cytokine interaction network offer a wealth of targets for therapeutic intervention.

Cross-References

- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Dendritic Cells in Atherosclerosis
- ▶ Immune-Mediated Mechanisms of the Metabolic Syndrome
- ▶ Interleukin-6
- ▶ Lymphocytes in Atherosclerosis
- ▶ Macrophages, Oxidative Stress, and Atherosclerosis
- ▶ Mechanisms of Endothelial Activation
- ▶ Nitric Oxide
- ▶ Platelets, Atherosclerosis, and Immunity
- ▶ Resolution of Inflammation
- ▶ Systemic Autoimmune Disease and Premature Atherosclerosis

References

Chen L, Frister A, Wang S, Ludwig A, Behr H, Pippig P, Li B, Simm A, Hofmann B, Pilowski C, Koch S, Buerke M, Rose-John S, Werdan K, Loppnow H. Interaction of vascular smooth muscle cells and monocytes by soluble factors synergistically enhances interleukin-6 and MCP-1 production. Am J Physiol. 2009;296:H987–96.

Chi H, Messas E, Levine RA, Graves DT, Amar S. Interleukin-1 receptor signaling mediates atherosclerosis associated with bacterial exposure and/or a high-fat diet in a murine apolipoprotein E heterozygote model - pharmacotherapeutic implications. Circulation. 2004;110:1678–85.

Dinarello CA. Biologic basis for interleukin-1 in disease. Blood. 1996;87:2095–147.

Eder C. Mechanisms of interleukin-1β release. Immunobiology. 2009;214:543–53.

Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol. 2006;6:508–19.

Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, Asano M, Moriwaki H, Seishima M. Lack of interleukin-1ß decreases the severity of atherosclerosis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol. 2003;23:656–60.

Kleemann R, Zadelaar S, Kooistra T. Cytokines and atherosclerosis: a comprehensive review of studies in mice. Cardiovasc Res. 2008;79:360–76.

Libby P. The vascular biology of atherosclerosis. In: Bonow RO, Mann DL, Zipes DP, Libby P, editors. Braunwald´s - heart disease. Philadelphia: Elsevier, Saunders; 2012. p. 897–913.

Loppnow H. Zytokine: Klassifikation, Rezeptoren, Wirkmechanismen. Internist. 2001;42:13–27.

Loppnow H, Libby P. Proliferating or interleukin-1-activated human vascular smooth muscle cells secrete copious interleukin-6. J Clin Invest. 1990;85:731–8.

Loppnow H, Bil R, Hirt S, Schönbeck U, Herzberg M, Werdan K, Rietschel ET, Brandt E, Flad H-D. Platelet-derived interleukin-1 induces cytokine production, but not proliferation of human vascular smooth muscle cells. Blood. 1998;91:134–41.

Loppnow H, Werdan K, Buerke M. Vascular cells contribute to atherosclerosis by cytokine- and innate-immunity-related inflammatory mechanisms. Innate Immun. 2008;14:63–87.

Loppnow H, Buerke M, Werdan K, Rose-John S. Contribution of vascular cell-derived cytokines to innate and inflammatory pathways in atherogenesis. J Cell Mol Med. 2011;15:484–500.

Nicola NA. Guidebook to cytokines and their receptors. Oxford: Oxford University Press; 1994.

Nieto FJ. Historical reflections on the inflammatory aspects. In: Wick G, Grundtmann C, editors. Inflammation and atherosclerosis. Wien: Springer; 2012. p. 1–18.

Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J. 2011;162:597–605.

Ross R. Atherosclerosis - an inflammatory disease. N Engl J Med. 1999;340:115–26.

Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev. 2006;86:515–81.

Thomson A, Lotze MT. The cytokine handbook. New York: Academic; 2003.

Tschopp J, Schroder K. NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? Nat Rev Immunol. 2010;10:210–5.

Autoantibodies in Rheumatoid Arthritis

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Synonyms

Autoimmunity; Autoimmune disease; Polyarthritis

Definition

Rheumatoid arthritis (RA) is a chronic disease of unknown cause marked by chronic joint inflammation. While RA is associated with a number of autoantibodies, the role of these antibodies in disease pathogenesis remains uncertain. Measures of RA-associated autoantibodies may be a source of diagnostic and prognostic information for individuals with suspected or established joint inflammation. Rheumatoid factor (RF) has long been the primary biomarker used by researchers and clinicians. However, recent studies confirm that anti-cyclic citrullinated peptide (anti-CCP) has higher specificity for rheumatoid arthritis than RF. In the future, it seems likely that additional autoantibodies will be discovered among individuals with RA. These could provide new insights into disease development and prove useful in the evaluation and treatment of individuals with rheumatoid arthritis or related disorders.

Introduction

In 1940, Waaler and Rose discovered the rheumatoid factor (RF) (Waaler 1940) and soon its association with rheumatoid arthritis (RA) provided compelling evidence of the autoimmune nature of RA. In addition, the RF became a common diagnostic test for patients with suspected rheumatoid arthritis or other rheumatic conditions. There was hope that determining the origin of RF production would unravel the pathogenic events leading to disease development.

Yet, many patients with RA lack significant quantities of RF in their blood. And, over time, other autoantibodies have been detected in patients with RA. The search for autoantibodies in RA continues in the hopes of advancing our understanding of disease pathogenesis, discovering preventive strategies, and improving our diagnostic and prognostic acumen. In the current era of early, aggressive treatment, the value of a highly sensitive and specific biomarker has never been higher.

At the current time, the presence of a single autoantibody cannot establish the diagnosis of RA or accurately predict its future development in an individual. Similarly, the absence of an autoantibody cannot rule out a current diagnosis of RA and a negative RF does not preclude its development in the future. Still, the presence or absence of autoantibodies in a person with polyarthritis can be helpful to the researcher, the clinician, and the patient.

Rheumatoid Factor (RF)

The RF is an antibody targeting the Fc portion of IgG. While a number of different RF immunoglobulin types (including IgM, IgA and IgG) can be detected in people with RA, it is the IgM subtype that is commonly called “RF” and that is usually measured in clinical practice. The strong association between RF and RA is clear and has been well established for decades. However, the reason some individuals produce RF, its physiologic function, and its role in disease pathogenesis remain unclear. RF does not appear to have a primary pathogenic role; that is, RF is not the cause of RA. However, it is associated with more severe disease. For example, RF-positive RA patients are more likely to have erosive and extra-articular disease than RF-negative RA patients.

While testing for RF is quite common in clinical practice, interpreting its results can be challenging because:

- The test is not standardized – There are many methods for measuring RF and different laboratories may provide different results on the same patient.
- A sizeable subset of patients with RA do not produce RF; estimates of "sero-negative" RA range from 10 % to 70 % (Shmerling and Delbanco 1991).
- The test is not specific for RA – Other conditions associated with chronic antigenic stimulation may be associated with RF. Examples include viral infections and subacute bacterial endocarditis (McInnes and Schett 2011).
- Genetic factors may drive RF production – As a result, people without RA but who have family members with RA may be more likely to be RF positive.
- RF may be present in rheumatic diseases other than RA, including most patients with Sjogren's syndrome and mixed connective tissue disease.

The 1958, 1987, and 2010 revised criteria for the classification of RA include the RF along with other clinical findings. Interestingly, the RF can be present years before clinical evidence of RA develops (Nielen et al. 2004).

Anti-Cyclic Citrullinated Peptide (Anti-CCP) Antibodies

Over the last decade, the high specificity of anti-CCP antibodies for RA has become clear (Nishimura et al. 2007; Whiting et al. 2010) and, as a result, testing patients with possible RA has become common. In fact, anti-CCP antibodies are the first highly specific markers for RA that are also sensitive enough to be clinically useful.

Anti-CCP antibodies target citrulline, an arginine molecule that has been posttranslationally modified by the action of peptidyl-arginine-deaminase. Two recent meta-analyses described the sensitivity and specificity of the anti-CCP antibody for RA as 57 % to 67 % and 96 % to 98 %, respectively (Nishimura et al. 2007; Whiting et al. 2010). Despite the high specificity of the test and the finding of citrullinated antigens in synovial tissue (De Rycke et al. 2005; Chang et al. 2005), there is no compelling evidence that anti-CCP antibodies are actually pathogenic. The absence of detectable anti-CCP antibody in the serum of a third or more of RA patients also suggests that the antibody is not pathogenic (or that RA is actually more than one disease). The observation that smoking and HLA-DR shared epitope genes are associated with anti-CCP positivity could be a clue to an interaction of environmental and genetic factors in the pathogenesis of seropositive RA (Klareskog et al. 2006; MacGregor et al. 2000).

Variations in anti-CCP antibody titer (and RF) may correlate with disease activity in some patients, but monitoring antibody levels is not routine or considered useful. However, the test does have prognostic significance: As with RF, anti-CCP-positive RA patients tend to have more active and erosive disease and tend to require more aggressive therapy (De Rycke et al. 2004).

In addition, anti-CCP-positivity may be predictive of response to specific treatments. For example, one study found that RA patients who are anti-CCP-positive are more likely to respond to anti-TNF therapy or rituximab than RA patients who are anti-CCP-negative (Braun-Moscovici et al. 2006; Lal et al. 2011).

The most common use of anti-CCP in clinical practice is for the diagnosis of RA; given the test's high specificity, it has high positive predictive value in settings of moderate to high pretest probability of RA (e.g., in setting of otherwise unexplained chronic, symmetric polyarthritis). As is true with RF, the anti-CCP antibody is included among the newly revised criteria for RA and the antibody can often be detected years before the clinical onset of disease (Aletaha et al. 2010).

Other Autoantibodies in RA

A number of other autoantibodies are detectable in patients with RA, including some that are more

Autoantibodies in Rheumatoid Arthritis, Table 1 Autoantibodies associated with rheumatoid arthritis other than RF and anti-CCP

Antibodies to citrullinated peptides other than anti-CCP		Sensitivity	Specificity	Comments
	Anti-mutated citrullinated vimentin	0.6 to 0.85	0.8 to 0.95	May correlate with radiographic erosions
	Anti-perinuclear factor and anti-keratin antibodies	0.49 to 0.85	0.7 to 0.99	May correlate with radiographic erosions and treatment-resistant disease; may precede symptoms
	Anti-Sa	0.45 to 0.7	0.8 to 0.96	May correlate with radiographic damage
	Anti-citrullinated fibrinogen	0.55 to 0.6	0.9 to 0.99	Similar operating characteristics to anti-CCP but not entirely overlapping
	Anti-citrullinated synthetic type I or type II collagen telopeptides	0.8	0.9	May increase risk of RA synergistically with anti-CCP antibodies
Anti-alpha-enolase		.25	Uncertain	May predict which patients with undifferentiated, early arthritis will eventually develop RA
Anti-p68 (BiP) antibodies		0.7	0.95	The p68 autoantigen could play a role in RA pathogenesis
p-ANCA		Uncertain	Uncertain	Occasional; perinuclear ANCA without anti-MPO specificity; possible correlation with joint damage
Antibodies to glucose phosphate isomerase (GPI)		Uncertain	Uncertain	May correlate with extra-articular manifestations of disease, including Felty's syndrome and rheumatoid vasculitis
Antibodies to PADI4		0.45	0.85 to 1.0	PADI4 is involved in the production of citrulline, another potential autoantigen

highly associated with another rheumatic disease. For example, up to 40% of patients with RA have a positive anti-nuclear antibody (ANA).

In general, their significance with respect to disease pathogenesis, diagnostic and prognostic utility, and implications for treatment is limited or uncertain. None of these are commonly used by clinicians outside of research settings for patients with suspected or established RA.

A few of these are listed in the Table 1.

Other Disease Associations

It is important to emphasize that the autoantibodies described in association with RA have variable specificity and so are found in other conditions. For example, up to a third of patients with SLE may be RF positive. False-positive RF results are usually found in low titer but exceptions do occur.

Despite the high specificity of the anti-CCP antibody, non-selective testing in settings of a low likelihood of RA will inevitably produce false-positive results. A number of studies have documented the presence of anti-CCP antibodies conditions other than RA, including infectious diseases (e.g., tuberculosis) and other rheumatic diseases (e.g., SLE, Sjogren's Syndrome, and psoriatic arthritis) (Elkayam et al. 2006; Maejima et al. 2010; Atzeni et al. 2008; Kakumanu et al. 2009).

Conclusions

It has become clear that RF is not the only autoantibody associated with RA. Some are likely "epiphenomena," perhaps, an indication of dysfunctional immune function. However,

others may be intimately involved in disease pathogenesis and may even be useful targets for treatment. Currently, no single antibody has been identified with perfect sensitivity and specificity for RA; however, combinations of autoantibodies may prove more useful than any one alone.

It is likely that in the future, new autoantibodies will be discovered among individuals with RA and that we will have a better understanding of how these antibodies can be helpful to providing insights into disease development and for the clinical evaluation and treatment of affected patients.

Cross-References

- ▶ Rheumatoid Arthritis, Clinical Features
- ▶ Rheumatoid Arthritis, Extra-articular Manifestations
- ▶ Rheumatoid Arthritis, Genetics
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham 3rd CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62:2569–81.

Atzeni F, Sarzi-Puttini P, Lama N, Bonacci E, Bobbio-Pallavicini F, Montecucco C, et al. Anti-cyclic citrullinated peptide antibodies in primary Sjögren syndrome may be associated with non-erosive synovitis. Arthritis Res Ther. 2008;10:R51.

Braun-Moscovici Y, Markovits D, Zinder O, Schapira D, Rozin A, Ehrenburg M, et al. Anti-cyclic citrullinated protein antibodies as a predictor of response to anti-tumor necrosis factor-alpha therapy in patients with rheumatoid arthritis. J Rheumatol. 2006;33:497–500.

Chang X, Yamada R, Suzuki A, Sawada T, Yoshino S, Tokuhiro S, et al. Localization of peptidylarginine deiminase 4 (PADI4) and citrullinated protein in synovial tissue of rheumatoid arthritis. Rheumatology (Oxford). 2005;44:40–50.

De Rycke L, Nicholas AP, Cantaert T, Kruithof E, Echols JD, Vandekerckhove B, et al. Synovial intracellular citrullinated proteins colocalizing with peptidyl arginine deiminase as pathophysiologically relevant antigenic determinants of rheumatoid arthritis-specific humoral autoimmunity. Arthritis Rheum. 2005;52:2323–30.

De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Meheus L, et al. Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. Ann Rheum Dis. 2004;63:1587–93.

Elkayam O, Segal R, Lidgi M, Caspi D. Positive anti-cyclic citrullinated proteins and rheumatoid factor during active lung tuberculosis. Ann Rheum Dis. 2006;65:1110–2.

Kakumanu P, Sobel ES, Narain S, Li Y, Akaogi J, Yamasaki Y, et al. Citrulline dependence of anti-cyclic citrullinated peptide antibodies in systemic lupus erythematosus as a marker of deforming/erosive arthritis. J Rheumatol. 2009;36:2682–90.

Klareskog L, Stolt P, Lundberg K, Källberg H, Bengtsson C, Grunewald J, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum. 2006;54:38–46.

Lal P, Su Z, Holweg CT, Silverman GJ, Schwartzman S, Kelman A, et al. Inflammation and autoantibody markers identify rheumatoid arthritis patients with enhanced clinical benefit following rituximab treatment. Arthritis Rheum. 2011;63:3681–91.

MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum. 2000;43:30–7.

Maejima H, Aki R, Watarai A, Shirai K, Hamada Y, Katsuoka K. Antibodies against cyclic citrullinated peptide in Japanese psoriatic arthritis patients. J Dermatol. 2010;37:339–45.

McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365:2205–19.

Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum. 2004;50:380–6.

Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, et al. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med. 2007;146:797–808.

Shmerling RH, Delbanco TL. The rheumatoid factor: an analysis of clinical utility. Am J Med. 1991;91:528–34.

Waaler E. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. Acta Path Microbiol Scand. 1940;17:172.

Whiting PF, Smidt N, Sterne JA, Harbord R, Burton A, Burke M, et al. Systematic review: accuracy of anti-citrullinated peptide antibodies for diagnosing rheumatoid arthritis. Ann Intern Med. 2010;152:456–64.

Autoantibodies to Endothelial Cells

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Synonyms

AECAs; Anti-endothelial cell antibodies

Definition

Endothelial cells form a single cell layer lining of the luminal wall of blood vessels. They are critical for the integrity of the vessel wall and in a prime location to interact with components circulating in the blood. Endothelial cells are capable of modulating the immune response by secretion of cytokines and by regulation of adhesion and transmigration of leukocytes. Antibodies targeted to endothelial cells are known as anti-endothelial cell antibodies (AECAs). AECAs were first described over 40 years ago and have since been detected in serum from patients with a wide range of medical conditions. AECAs represent a heterogeneous group of antibodies directed against a range of endothelial cell antigens. The characterization of antigenic targets of AECAs is incomplete, but known targets include membrane antigens, ligand-receptor complexes, and planted molecules which are circulating proteins that have become adhered to the endothelium. AECAs can also be found in healthy people. These "natural endothelial cell antibodies" have physiological functions increasing the anti-inflammatory effects of endothelial cells.

Detection of AECAs

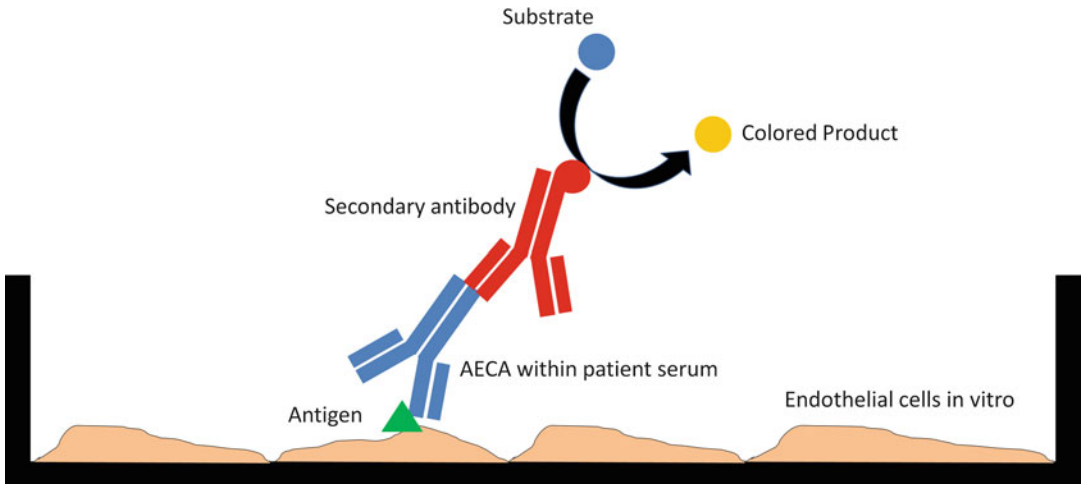
The reported incidence and prevalence of AECAs varies widely, partly due to differences in assays used to quantify them and a lack of gold standard method of detection (Youinou et al. 1995). The majority of studies have relied on cell-based enzyme-linked immunosorbent assays (ELISA) with human umbilical vein endothelial cells (HUVEC) as a detection system (Fig. 1).

The fixation process utilized in cell-based ELISA results in permeabilization of the endothelial cells, exposing intracellular antigens as well as surface membrane antigens to the test antibody leading to false-positive results. Flow cytometry analysis of unfixed endothelial cells detects more specifically AECAs directed against surface antigens (Westphal et al. 1994). Other techniques including indirect immunofluorescence, immunoprecipitation in conjunction with Western blotting, fluorescence-activated cell sorting (FACS), and radioimmunoassay have also been utilized to detect AECAs. Each technique has its own limitations.

AECAs can be distinguished on the basis of their reactivity to the endothelium of capillaries and large vessels (Praprotnik et al. 2001). Further, the binding affinity of AECAs varies depending on the endothelial cell target used, with differences between cell donors and cell line localization. Granulomatosis with polyangiitis (Wegener's) is associated with organ-specific AECAs. In a study by Holmen et al., the reactivity of AECA to endothelial cells depended on the origin of the endothelial cell line; there was a high incidence of AECAs reactive with human kidney microvascular endothelial cells (71 %) but lower reactivity to nasal endothelial cells (61 %), pulmonary microvascular endothelial cells (25 %), and HUVEC (7 %) (Holmen et al. 2004).

Incidence of AECAs

AECAs have been associated with a wide range of diseases. The most evidence exists for an association with antiphospholipid syndrome (discussed in detail later) and the systemic vasculitides, a group of disorders characterized by inflammation within the vascular wall. AECAs have been described in large-, medium-, and small-vessel vasculitides including giant cell



Autoantibodies to Endothelial Cells, Fig. 1 Indirect ELISA. Endothelial cells are grown in vitro until confluent. Patient serum is added. AECA present within the serum binds to an unidentified antigenic target present on the endothelial cells. A secondary antibody is applied which binds to bound AECA. Unbound antibody is washed away. This secondary antibody has an enzyme conjugated to it. Added substrate changes color in the presence of the conjugated enzyme. The higher the concentration of AECA within the sample, the greater the concentration of bound enzyme and the stronger the color change. This can be measured using a spectrometer, thereby quantifying the concentration of AECA within the original sample

arteritis, Takayasu arteritis, polyarteritis nodosum, Kawasaki disease, Henoch-Schonlein purpura, and the antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides, namely, granulomatosis with polyangiitis (Wegener's) and microscopic polyangiitis. In granulomatosis with polyangiitis (Wegener's), AECAs are found in 19–71 % of people (reviewed in Savage and Williams (2007)). The wide-ranging estimates of prevalence are likely in part to be due to differences in the detection system used.

AECAs are also reported in association with other systemic autoimmune and connective tissue disorders. Estimates of prevalence are widespread. They have been reported in up to 90 % of those with systemic lupus erythematosus (SLE) and up to 68 % of those with rheumatoid arthritis, with increased prevalence in the setting of rheumatoid arthritis in association with vasculitis. In addition, AECAs are associated with Behcet's syndrome, Sjogren's syndrome, and systemic sclerosis.

Vascular injury has also been implicated in diseases not classically thought of as vasculitides. AECA levels are elevated in inflammatory bowel disease in some studies. In ulcerative colitis, AECA levels are correlated with circulating levels of von Willebrand factor, a marker of vascular injury. AECA levels are also increased in a series of miscellaneous disorders including chronic obstructive pulmonary disease, insulin-dependent diabetes mellitus, multiple sclerosis, masked hypertension, previous myocardial infarction, peripheral arterial disease, preeclampsia, thrombotic thrombocytopenic purpura, heparin-induced thrombocytopenia, and kidney allograft rejection.

AECA Targets

Given the wide range of diseases associated with AECAs, it is likely that the targets for AECAs are diverse. Antigenic targets may be constitutively expressed or modulated by cytokines in the context of inflammation or infection, for example, CMV infection. Planted molecules which bind to the surface of endothelial cells can also act as targets for AECAs, for example, DNA in SLE, myeloperoxidase and proteinase 3 in

ANCA-associated vasculitis, and β2 glycoprotein I in antiphospholipid syndrome. In many cases, the specific antigenic target for AECAs is unknown. Recent hypothesized targets include heat shock protein 60 (hsp60), peroxiredoxin 2 in systemic vasculitis, topoisomerase I and CENP-B in systemic sclerosis, alpha-enolase in Behcet's syndrome, and tropomyosin and T-plastin in Kawasaki disease. Heparin sulfate proteoglycans or heparin-like molecules have been suggested as a target for AECAs. The antibodies from patients with heparin-induced thrombocytopenia recognize platelet factor 4 bound to heparin, stimulating endothelial cells to produce tissue factor and bind platelets.

Although the presence of AECAs have been detected in a number of conditions, the clinical relevance is less clear. Are AECAs pathogenic or are they formed secondary to endothelial damage? Do they have a role in promoting a proinflammatory environment, or do they exhibit more direct cytotoxic effects? In vitro studies give useful insight into potential mechanisms of pathogenicity of AECAs; clinical studies provide evidence for the role of AECAs in causing disease.

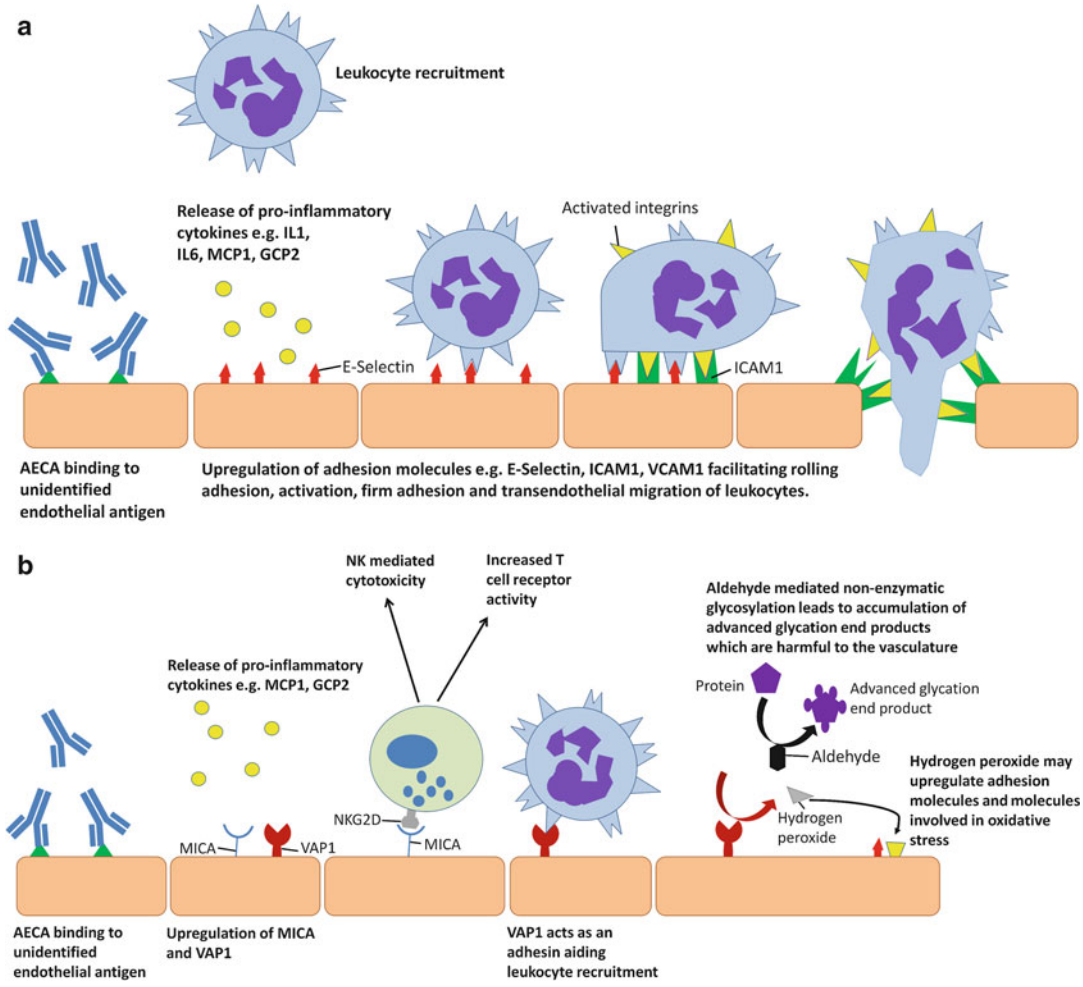
Potential Mechanisms of Pathogenicity

AECAs may act via direct antigen-specific mechanisms, for example, through activation of cell signaling cascades resulting in leukocyte activation and recruitment, or via non-antigen-specific pathways, for example, triggering antibody-dependent cellular cytotoxicity (ADCC) and apoptosis.

Effects of AECAs

AECAs may have direct functional effects, triggering cell signaling cascades on binding to endothelial cell antigens resulting in upregulation of adhesion molecules, recruitment of leukocytes, and secretion of proinflammatory cytokines, with potential to further potentiate the process via autocrine effects (Papa et al. 1999) (Fig. 2a). Immunoglobulin G from patients with SLE and systemic vasculitis upregulates adhesion molecule expression (intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin) and leukocyte adhesion in vitro, mediated via interleukin-1 and an unidentified cytokine released from endothelial cells (Carvalho et al. 1999). Binding of AECA from granulomatosis with polyangiitis (Wegener's) patients to human kidney microvascular endothelial cells (HKMEC) elicited a calcium flux associated with secretion of monocyte chemotactic protein-1 (MCP-1) and granulocyte chemotactic protein-2 (GCP-2) (Fig. 2b). MCP-1 and GCP-2 are cytokines that mediate monocyte, T cell, and neutrophil infiltration and upregulation of the ligands MHC class I-related antigen A (MICA) and vascular adhesion protein-1 (VAP-1). MICA is a ligand for the co-stimulatory receptor NKG2D which is expressed on natural killer (NK) cells, CD8 T cells, and $\gamma\delta^+$ T cells and is involved in increasing T cell receptor activity and cell-mediated toxicity of NK cells. VAP-1 has roles in chemotaxis and oxidative stress through mediating production of hydrogen peroxide, aldehydes, and ammonium. Renal biopsy specimens from patients with active granulomatosis with polyangiitis (Wegener's) had strong expression of MICA and VAP-1 as well as evidence of infiltrating cytotoxic $CD8^+$ and $\gamma\delta^+$ T cells and cells expressing the MICA ligand NKG2D, suggesting a role for AECAs in initiating leukocyte recruitment in ANCA-associated vasculitis (Holmen et al. 2007).

AECAs may act in this direct way at concentrations below the limit of detection by conventional immunohistochemistry, offering a possible mechanism of pathogenesis in pauci-immune conditions. Monoclonal AECA IgG generated by hybridoma formation with human SLE cells induces a proadhesive and proinflammatory endothelial phenotype through nuclear factor kappa B (NF-κB) activation, with involvement of an autocrine loop of interleukin-1β secretion. Endothelial cell activation was found to be dose dependent. E-selectin was upregulated at lower concentrations of AECA than ICAM-1 or interleukin-6, perhaps facilitated by the autocrine effects of cytokines produced by endothelial cells (Yazici et al. 2001). Absence of AECAs on



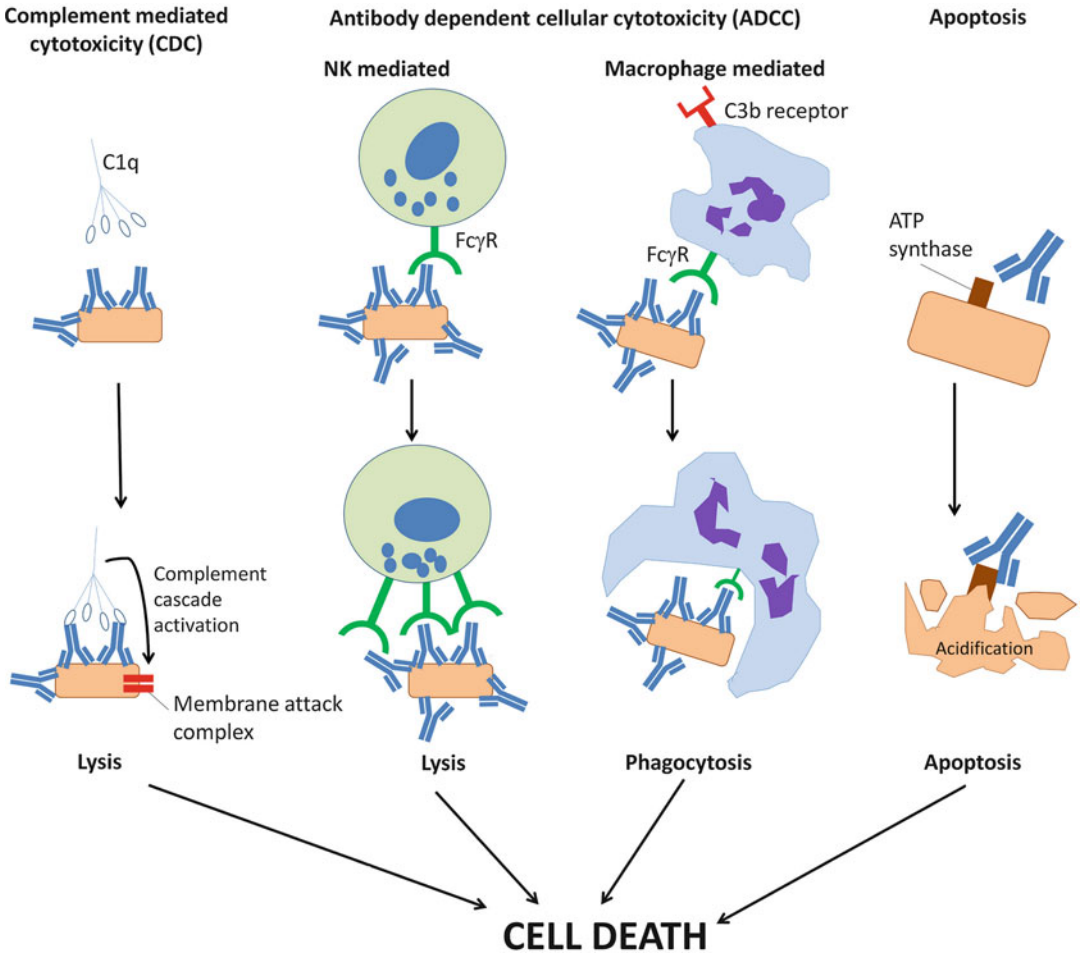
Autoantibodies to Endothelial Cells, Fig. 2 Direct effects of AECAs. (**a**) AECA binding to endothelial cells causes release of proinflammatory cytokines and upregulation of adhesion molecules. As a result, leukocytes are recruited and able to progress through the leukocyte adhesion cascade, resulting in transendothelial migration and accumulation at the site of inflammation. (**b**) Direct effects of AECAs. The cytokines MCP1 and GCP2 cause upregulation of MHC class I-related antigen A (*MICA*) and vascular adhesion protein-1 (*VAP1*). MICA is a ligand for NKG2D, a co-stimulatory receptor expressed by NK cells, CD8 T cells, and γδ T cells. Engagement leads to NK-mediated cytotoxicity and increased T cell receptor activity. VAP1 is both an adhesin and an enzyme involved in producing potent inflammatory mediators, for example, hydrogen peroxide, aldehyde, and ammonium

immunochemistry can also be explained by low avidity binding via the $F(Ab)_2$ portion of the immunoglobulin.

Indirect effects of AECAs

Complement-Mediated Lysis and Antibody-Dependent Cellular Cytotoxicity

AECAs can also cause damage via complement-mediated lysis or antibody-dependent cellular cytotoxicity in vitro (Fig. 3). However, there are questions over the in vivo relevance given the high effector/target cell ratios required. In SLE, complement-fixing AECAs are capable of mediating platelet adhesion and stimulating the release of tissue factor from endothelial cells, promoting thrombus formation (Cines et al. 1984). Recent evidence suggests that complement activation is important even in the absence of



Autoantibodies to Endothelial Cells, Fig. 3 Indirect effects of AECAs. Opsonization of cells by AECA marks them as a target for phagocytosis, NK-mediated antibody-dependent cellular cytotoxicity (*ADCC*), and complement-mediated cytotoxicity. AECAs can cause apoptosis directly via an interaction with surface ATP synthase

demonstrable immune complex deposition in pauci-immune vasculitis. The neutrophil C5a receptor may even prove to be a useful therapeutic target in ANCA-associated vasculitis.

Induction of Apoptosis

AECAs are capable of inducing endothelial cell apoptosis. Binding of AECA to endothelial cell surface ATP synthase, the endogenous receptor for heat shock protein 60 (hsp60), induced intracellular acidification which is known to induce endothelial cell death and trigger inflammation (Jamin et al. 2005). Normally apoptotic cells are cleared by macrophages in a noninflammatory manner. AECA opsonization of apoptotic cells may increase macrophage phagocytosis and trigger a further inflammatory response from macrophages through engagement of Fc receptors.

Do AECAs Have a Role in Disease Pathogenesis?

Association studies do not prove that AECAs are pathogenic and that they cause disease. AECAs may merely be associated with disease, an irrelevant epiphenomenon related to

endothelial damage. AECAs may potentiate endothelial damage even if they are not the primary cause of it, for example, by amplifying endothelial injury initiated by proteolytic enzymes released from neutrophils following activation by antineutrophil antibodies in ANCA-associated vasculitis.

Animal models provide some evidence for pathogenicity. Three out of ten mice injected with ANCA-depleted IgG derived from a granulomatosis with polyangiitis (Wegener's) patient developed mouse antihuman AECAs and subsequently perivascular lymphoid cell infiltrates as well as deposition of IgG at the outer part of blood vessel walls within kidney and lung. The lungs and kidneys from AECA-negative mice were free of any inflammatory cell infiltration (Damianovich et al. 1996).

Clinical human studies also support a role in disease pathogenesis. Several studies have correlated the presence and levels of AECAs with markers of disease activity and organ damage. AECAs may be an independent risk factor for disease relapse in ANCA-associated vasculitis. However, this correlation is not universal; the largest study to date looking at 173 granulomatosis with polyangiitis (Wegener's) patients enrolled in a clinic therapeutic trial found no correlation between the presence of AECAs measured using HUVEC in a baseline serum sample with markers of disease activity (Sebastian et al. 2007). AECAs have been isolated from patients with ANCA-negative pauci-immune glomerulonephritis, suggesting perhaps a role for AECAs in the absence of ANCA. Furthermore in this patient group, different AECAs were associated with different clinical presentations of disease.

In some cases, presence of AECAs appears to correlate with a particular phenotype of disease. For example, in Behcet's syndrome, AECAs are associated with previous CNS involvement. In sarcoidosis, AECAs are associated with multiple lesions and requirement for corticosteroid therapy. In SLE, AECAs are associated with lupus nephritis and pulmonary hypertension. In systemic sclerosis, they are associated with severe digital ischemia and pulmonary arterial hypertension; prevalence rates are higher in those with diffuse compared to those with limited scleroderma.

Antiphospholipid Antibodies

Antiphospholipid antibodies (aPL) are a well-characterized form of AECA. Antiphospholipid syndrome is a condition characterized by a prothrombotic state causing venous and arterial thrombosis in the presence of aPL. It is often diagnosed following ischemic stroke or thromboembolic disease affecting the young or following recurrent miscarriage. Antiphospholipid syndrome can be described as primary in the absence of another autoimmune disease or secondary to a preexisting autoimmune disease, most commonly SLE.

The main antigenic target for aPL is β2 glycoprotein I (β2GPI), a protein planted on endothelial cells. β2GPI is a cationic protein that displays high binding affinity to endothelial cells via electrical interactions with cell membrane structures such as heparin sulfate, via interactions with the annexin-2 receptor, and potentially also via cell membrane receptors for lipoproteins, given β2GPI's role in lipid transport. Affinity-purified antibody against β2GPI isolated from patients with aPL syndrome binds to endothelial cells. Binding is reduced by removal of β2GPI in vitro and restored in a dose-dependent manner by exogenous human β2GPI.

Treatment with aPL induces endothelial cell activation, with increased expression of adhesion molecules (VCAM-1 and E-selectin); increased tissue factor transcription, expression, and function; and increased interleukin-6 production. Tissue factor acts as a receptor for activated factor VII (VIIa), increasing its activity within the coagulation cascade. Under normal circumstances, circulating factor VII is not exposed to tissue factor and has low activity. Vascular injury and inflammatory mediators, for example, tumor necrosis factor alpha (TNF-α) and bacterial lipopolysaccharide, can induce expression of tissue factor, converting the endothelium into a procoagulant state. Tissue factor expression on

the endothelium has also been described in association with sepsis and malignancy. Inappropriate expression of tissue factor therefore may underpin the thrombotic process seen in antiphospholipid syndrome and other conditions.

Thus, aPL – defined as antibodies are capable of activating endothelial cells in vitro and in vivo and appear to be implicated in the thrombogenic state of antiphospholipid syndrome. Experimental animal models have supported the in vivo pathogenic role of anti-β2GPI antibodies in both thrombosis and fetal loss. Anti-β2GPI antibodies therefore represent a class of AECAs where the target, potential signaling pathways, and pathogenic mechanisms are well defined (Fig. 4).

Alternative AECA targets may also play a role in the pathogenesis of antiphospholipid syndrome. Autoantibodies to heparin sulfate inhibit formation of antithrombin III-thrombin complexes, a natural anticoagulant, thus favoring a thrombophilic state. AECAs also interfere in apoptotic cell clearance (Graham et al. 2009). Clearance of procoagulant apoptotic cells by healthy endothelial cells is inhibited, while opsonization by AECA enhances Fc-mediated uptake by professional phagocytes. This change results in increased thrombin generation and a proinflammatory, procoagulant state.

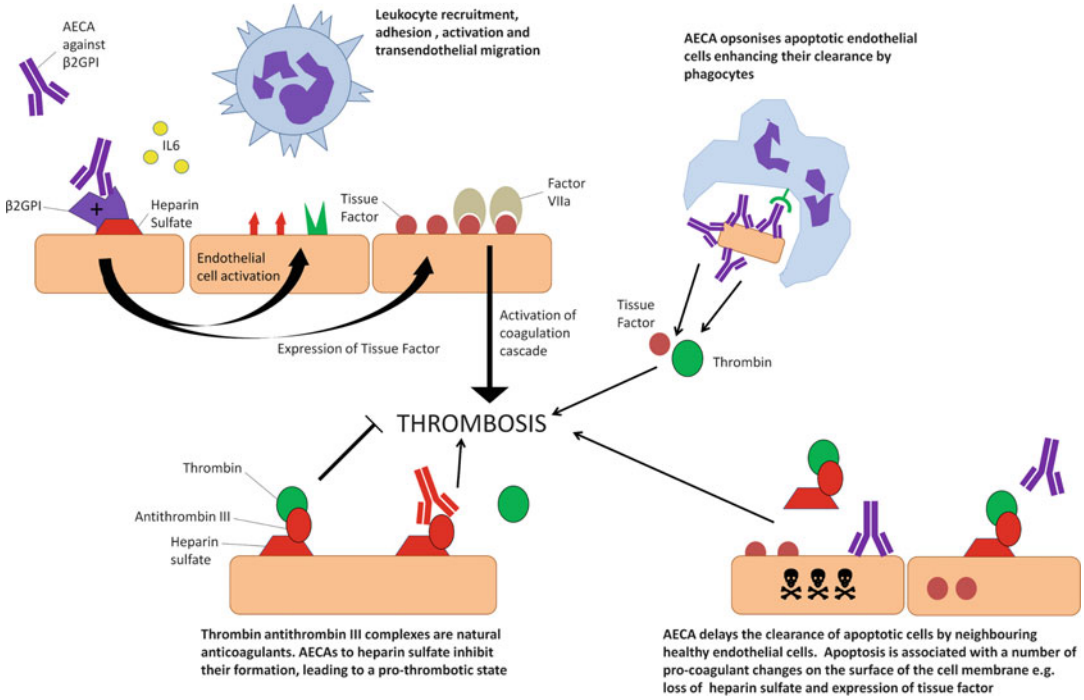
AECAs and Cardiovascular Impact

Patients with autoimmune disease such as rheumatoid arthritis, SLE, and systemic vasculitis have increased risk of cardiovascular disease. Cardiovascular disease is the leading cause of mortality in these conditions. Chronic inflammation is thought to be a risk factor for accelerated atherosclerosis by promoting vascular endothelial dysfunction. Classical risk factors for atherosclerosis precipitate endothelial dysfunction; hypertension is associated with increased shear stress; cigarette smoking is associated with free radical damage. AECAs may contribute by causing endothelial injury, promoting a proinflammatory, proadhesive endothelial cell phenotype and altering the metabolism of lipoproteins involved in atherogenesis (reviewed in Narshi et al. (2011)). The resultant endothelial dysfunction may lead to atherosclerotic plaque formation and subsequent cardiovascular events. The importance of adhesion molecule expression is shown by a reduction of plaque lesions in atherosclerosis-prone apolipoprotein E-deficient mice that are also deficient in E-selectin (Dong et al. 1998). Elevated levels of soluble VCAM-1 in the systemic circulation have been identified in humans with coronary artery disease.

Antiphospholipid antibodies have been particularly studied in relation to accelerated atherosclerosis. ß2GPI is present in human atherosclerotic plaques, and adoptive transfer of ß2GPI reactive lymphocytes enhances early atherosclerosis in LDL receptor-deficient mice. Antiphospholipid antibodies may enhance atherosclerosis via oxidative modification of lipoproteins, for example, low-density lipoprotein, and by promoting the uptake of lipoproteins by macrophage scavenger receptors. In clinical studies, the relevance of aPL antibodies in atherosclerotic disease is hotly debated. In a prospective study of 182 SLE patients, presence of aPL, elevated markers of endothelial activation (von Willebrand factor (vWf), soluble vascular cellular adhesion molecule-1 (sVCAM-1)), and fibrinogen were found to be predictors for first cardiovascular event in age-adjusted Cox regression analysis. The association with positive aPL was maintained in a multivariable Cox model (Gustafsson et al. 2009).

AECAs have been shown to be associated with ischemic heart disease in the absence of autoimmune disease, with increased prevalence of AECA positivity in those undergoing coronary angiography for ischemic heart disease compared to controls, where those with clinical and/or laboratory features of SLE, systemic sclerosis, or vasculitis were excluded. There was also an association with severity of symptoms and restenosis rates.

Statins inhibit anti-β2GPI antibody-mediated endothelial cell activation, downregulating the expression of adhesion molecules. These drugs, proven to be beneficial in cardiovascular disease, may be acting in part through modulation of AECA pathogenicity.



Autoantibodies to Endothelial Cells, Fig. 4 AECAs in antiphospholipid syndrome. AECAs are pathogenic in antiphospholipid syndrome. β2GPI is a cationic protein that is planted on the endothelial surface and bound to endothelial proteins, for example, heparin sulfate, via electrical interactions. AECAs against β2GPI activate endothelial cells, leading to increased leukocyte recruitment. AECAs promote a prothrombotic environment through upregulation of tissue factor expression, inhibition of the natural anticoagulant action of thrombin-antithrombin III, impaired clearance of apoptotic cells, and increased phagocyte activity. This prothrombotic state underpins the clinical features of antiphospholipid syndrome

AECA in Transplantation

Accelerated cardiovascular disease is also a problem following transplantation. AECAs have been associated with coronary artery disease following cardiac transplantation. Chronic allograft rejection has histological findings of narrowing of muscular arteries or obliterative arteriopathy. The vascular endothelium of transplanted organs represents an important target of allograft-directed immune responses, and endothelial cell injury is paramount in chronic allograft rejection. Both cellular and humoral arms of the immune system have been implicated. AECAs have been associated with acute rejection, chronic rejection, and decreased allograft survival in renal transplantation.

The role of AECAs directed against antigens other than human leukocyte antigen (HLA) in kidney allograft rejection is being increasingly studied. Sun et al. used HUVEC as a substrate for a cell-based ELISA detecting non-HLA antibodies pre- and posttransplantation. The presence of preexisting AECAs was not associated with either an increased risk of rejection or a detrimental impact on recipient/graft survival. However, de novo AECAs appearing posttransplantation in patients who did not have AECAs pretransplantation were associated with a higher risk of early rejection, which tended to be more severe. There were histological similarities to classic antibody-mediated rejection despite the absence of C4d deposition (Sun et al. 2011). However, critics claim that the study did not prove that AECA reactivity was not due to HLA antibodies reactive with the nonspecific HUVEC assay used. XM-ONE is a method of flow cytometry crossmatch using donor precursor endothelial

cells as target cells. Using this technique, Xavier P et al. showed an association of AECAs with early acute rejection following renal transplantation. In addition, they identified a subgroup of patients experiencing rejection (19/43) who had AECAs but not HLA antibodies. A positive AECA result in the setting of rejection was significantly associated with a poor prognosis, with 20/31 going on to develop progressive graft dysfunction (Xavier et al. 2011). The majority of AECA-positive patients (51 %) did not experience rejection, perhaps due to the absence of target antigens on the renal vascular endothelium.

Infectious Agents and AECAs

AECAs may be driven by the presence of microorganisms, for example, cytomegalovirus (CMV). Most viral infections can induce polyclonal B cell activation, triggering autoantibody production as well as a viral-specific antibody response. CMV infection upregulates adhesion and major histocompatibility complex (MHC) molecule expression on CMV-infected endothelial and immune cells and increases their cytokine secretion. CMV infection also modifies the peptides expressed on HLA class I and II molecules; some CMV peptides display molecular mimicry with alloantigens; additionally, neoantigens are produced during CMV infection and may be expressed on endothelial cells.

Increased levels of AECAs have been reported in a small group of cardiac and renal transplant recipients during and after CMV infection. CMV infection is known to be associated with both acute and chronic allograft rejection. CMV infection has been implicated more widely in the atherosclerotic process, with studies associating levels of CMV antibodies with patients with vascular disease and isolation of CMV antigens from atherosclerotic plaques. Could this be in part due to CMVs role in AECA formation?

There are also associations between AECAs and other infectious diseases. In dengue fever, the host immune response contributes to endothelial cell damage in addition to direct viral damage. Antibodies against dengue virus nonstructural protein 1 (NS1) cross-react with endothelial cells, inducing endothelial cell apoptosis and cell lysis in the presence of complement. The resulting vascular leak, endothelial dysfunction, and hemorrhagic diathesis may contribute to the life-threatening complications of dengue hemorrhagic fever and dengue shock syndrome (Lin et al. 2003).

Conclusion

There is growing evidence to suggest an association between AECAs and a wide range of diseases. The difficulties in measuring AECAs, lack of gold standard technique, and incomplete identification of targets have complicated this area of research, making it difficult to draw firm conclusions.

In vitro models have enabled identification of some of the downstream effects of AECAs. Endothelial cell activation and subsequent upregulation of adhesion molecules, tissue factor, and secretion of proinflammatory cytokines provide a mechanism by which AECAs could mediate their effects in autoimmune disease and cardiovascular disease.

It remains unclear if antibodies occur prior or secondary to endothelial cell damage. Perhaps this distinction is somewhat academic if the antibodies contribute by amplifying the disease process. There is a pressing need to identify the antigenic targets of AECAs. Until they are identified and antigen-specific ELISAs are developed, it will not be possible to determine the true incidence of AECAs in disease states and health. As more specific antigen targets are identified, the study of direct antibody effects will become possible and the role in pathogenesis will become more clear opening up opportunities for therapeutic intervention.

Cross-References

- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Mechanisms of Endothelial Activation
- ▶ Systemic Autoimmune Disease and Premature Atherosclerosis

References

Carvalho D, Savage CO, Isenberg D, Pearson JD. IgG anti-endothelial cell autoantibodies from patients with systemic lupus erythematosus or systemic vasculitis stimulate the release of two endothelial cell-derived mediators, which enhance adhesion molecule expression and leukocyte adhesion in an autocrine manner. Arthritis Rheum. 1999;42(4):631–40.

Cines DB, Lyss AP, Reeber M, Bina M, DeHoratius RJ. Presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus. J Clin Invest. 1984;73(3):611–25.

Damianovich M, Gilburd B, George J, Del Papa N, Afek A, Goldberg I, et al. Pathogenic role of anti-endothelial cell antibodies in vasculitis. An idiotypic experimental model. J Immunol. 1996;156(12):4946–51.

Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, Wagner DD. The combined role of P- and E-selectins in atherosclerosis. J Clin Invest. 1998;102(1):145–52.

Graham A, Ford I, Morrison R, Barker RN, Greaves M, Erwig LP. Anti-endothelial antibodies interfere in apoptotic cell clearance and promote thrombosis in patients with antiphospholipid syndrome. J Immunol. 2009;182(3):1756–62.

Gustafsson J, Gunnarsson I, Borjesson O, Pettersson S, Moller S, Fei GZ, et al. Predictors of the first cardiovascular event in patients with systemic lupus erythematosus – a prospective cohort study. Arthritis Res Ther. 2009;11(6):186.

Holmen C, Christensson M, Pettersson E, Bratt J, Stjarne P, Karrar A, et al. Wegener's Granulomatosis is associated with organ-specific antiendothelial cell antibodies. Kidney Int. 2004;66(3):1049–60.

Holmen C, Elsheikh E, Christensson M, Liu J, Johansson AS, Qureshi AR, et al. Anti endothelial cell autoantibodies selectively activate SAPK/JNK signalling in Wegener's granulomatosis. J Am Soc Nephrol: JASN. 2007;18(9):2497–508.

Jamin C, Dugue C, Alard JE, Jousse S, Saraux A, Guillevin L, et al. Induction of endothelial cell apoptosis by the binding of anti-endothelial cell antibodies to Hsp60 in vasculitis-associated systemic autoimmune diseases. Arthritis Rheum. 2005;52(12):4028–38.

Lin CF, Lei HY, Shiau AL, Liu CC, Liu HS, Yeh TM, et al. Antibodies from dengue patient sera cross-react with endothelial cells and induce damage. J Med Virol. 2003;69(1):82–90.

Narshi CB, Giles IP, Rahman A. The endothelium: an interface between autoimmunity and atherosclerosis in systemic lupus erythematosus? Lupus. 2011;20(1):5–13.

Papa ND, Raschi E, Moroni G, Panzeri P, Borghi MO, Ponticelli C, et al. Anti-endothelial cell IgG fractions from systemic lupus erythematosus patients bind to human endothelial cells and induce a pro-adhesive and a pro-inflammatory phenotype in vitro. Lupus. 1999;8(6):423–9.

Praprotnik S, Blank M, Meroni PL, Rozman B, Eldor A, Shoenfeld Y. Classification of anti-endothelial cell antibodies into antibodies against microvascular and macrovascular endothelial cells: the pathogenic and diagnostic implications. Arthritis Rheum. 2001;44(7):1484–94.

Savage CO, Williams JM. Anti endothelial cell antibodies in vasculitis. J Am Soc Nephrol: JASN. 2007;18(9):2424–6.

Sebastian JK, Mahr AD, Ahmed SS, Stone JH, Romay-Penabad Z, Davis JC, et al. Antiendothelial cell antibodies in patients with Wegener's granulomatosis: prevalence and correlation with disease activity and manifestations. J Rheumatol. 2007;34(5):1027–31.

Sun Q, Cheng Z, Cheng D, Chen J, Ji S, Wen J, et al. De novo development of circulating anti-endothelial cell antibodies rather than pre-existing antibodies is associated with post-transplant allograft rejection. Kidney Int. 2011;79(6):655–62.

Westphal JR, Boerbooms AM, Schalwijk CJ, Kwast H, De Weijert M, Jacobs C, et al. Anti-endothelial cell antibodies in sera of patients with autoimmune diseases: comparison between ELISA and FACS analysis. Clin Exp Immunol. 1994;96(3):444–9.

Xavier P, Aires P, Sampaio S, Mendes C, Monteiro M, Alves H, et al. XM-ONE detection of endothelium cell antibodies identifies a subgroup of HLA-antibody negative patients undergoing acute rejection. Transpl Proc. 2011;43(1):91–4.

Yazici ZA, Raschi E, Patel A, Testoni C, Borghi MO, Graham AM, et al. Human monoclonal anti-endothelial cell IgG-derived from a systemic lupus erythematosus patient binds and activates human endothelium in vitro. Int Immunol. 2001;13(3):349–57.

Youinou P, Meroni PL, Khamashta MA, Shoenfeld Y. A need for standardization of the anti-endothelial-cell antibody test. Immunol Today. 1995;16(8):363–4.

Autoimmune Blistering Diseases

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Autoimmune Bullous Disease Introduction

The bullous skin diseases, although uncommon, may have substantial morbidity. Coupled

Autoimmune Blistering Diseases, Table 1 Autoimmune Bullous diseases

Disease	Direct IF	Isotype	Target antigens	Location
Subcorneal				
Pemphigus foliaceus	Intercellular	IgG	Desmoglein-1	Desmosome
	Greater in upper epidermis		Desmocollins	
	C3 may be present		Other plakins	
Pemphigus erythematosus	Intercellular	IgG	Desmoglein-1	Desmosome
	Greater in upper epidermis			
	C3 is present			
	Granular deposition in BMZ			
Intraepidermal				
Pemphigus vulgaris	Intercellular	IgG	Desmoglein-3	Desmosome
	Greater in lower epidermis	Rarely, few IgA	Desmoglein-1	
	C3 often present	Rarely, few IgM	Desmocollins	
			Desmoplakin	
			E-cadherin	
			Other non-cadherin targets	
Pemphigus vegetans	Intercellular	IgG	Desmoglein-3	Desmosome
			Other non-Desmocollins 1	
			Other non-Desmocollins 2	
Paraneoplastic pemphigus	Intercellular	IgG	Envoplakin	Desmosome
	Other non-May have subepidermal component		Periplakin	
	Other non-C3 may be present		Desmoplakin 1	
			Desmoplakin 2	
			Plectin	
			BP230	Hemidesmosome
Subepidermal				
Bullous pemphigoid	BMZ, linear	IgG	BP 230	Hemidesmosome
	C3 present, may see only C3	Rarely, few IgA	BP 180	
		Rarely, few IgM		
Mucous membrane pemphigoid	BMZ, linear	IgG	BP 180	Hemidesmosome
	C3 present	Rarely, few IgA	Laminin 5	
			α6β4 integrin	
			BP 230	
			Collagen VII	
Linear IgA disease	BMZ, linear	IgA	BP180	Hemidesmosome
	C3 may be present	Rarely, few IgG	(Shed ectodomain)	
		Rarely, few IgA	LAD285	
			BP230	
			Collagen VII	

with the clinical presentation, these autoimmune disorders are characterized by their immunopathology and histopathology. Pathogenic autoantibodies bind to antigens with adhesion functions within the epidermis or dermis. The level of split within the skin or mucous membranes dictates the specific morphology. The target antigens are components of intercellular desmosomes or the adhesion complex, which is the basement membrane zone (BMZ) functional unit. However, target antigens and specific epitopes may be found with more than one disorder. The overlap between blistering disorders can make a definitive diagnosis challenging. Even when the specific target antigen is known, the exact pathogenesis for many bullous diseases is not fully understood.

Immunofluorescence (IF), along with the histopathologic features, has become the mainstay of diagnosis and has helped determine the specific pathogenic antigens for many of the autoimmune bullous disorders. Direct IF displays the deposition site of immunoglobulins, complement, and fibrinogen, whereas indirect IF of the serum detects circulating antibodies.

Depending on the target antigen, the level of split may be subcorneal (beneath the stratum corneum), intraepidermal (within the stratum spinosum), or subepidermal (at the dermal-epidermal junction). Subcorneal and intraepidermal blisters rupture easily and are classically described as "flaccid," as opposed to "tense" subepidermal blisters.

The autoimmune blistering disorders will be presented here sequentially by blister depth in the skin, from more superficial to deep (Table 1).

Cross-References

► Subepidermal Blistering Diseases: Bullous Pemphigoid

Autoimmune Heart Disease: Animal Models

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Definition

Autoimmune heart disease defines any structural or functional alteration of heart tissue due to aberrant immune responses against cardiac self-antigens, which impairs the capacity of the heart to maintain sufficient cardiac output to meet metabolic demands of the body. The term autoimmune myocarditis specifies cardiac inflammation due to an autoimmune response. Of note, autoimmune heart disease can also be part of a general self-reactive process involving other organ systems as well. This entry, however, focuses exclusively on animal models, which (1) mirror heart-specific autoimmunity only, and (2) on animal models in which self-antigen exposure but not infection trigger autoimmunity.

Introduction

During the past several years, autoimmune mechanisms had been increasingly recognized to be involved in the development of almost all cardiac diseases, such as dilated or structural cardiomyopathy, valvular heart disease, coronary arterial disease, hypertensive heart failure, and even conduction disturbances. In the following entry, however, I focus specifically on animal models of autoimmune myocarditis and postinflammatory cardiomyopathy.

Despite the fact that myocarditis can be observed in many species, such as anthropoid apes, rabbits, dogs, cats, and guinea pigs, current

animal research activities rely mostly on mouse and rat models. This, however, was not the case in the past: one of the first landmark papers describing a "filter-passing agent" isolated from the chest cavity of a male gibbon who died from myocarditis reported myocarditis-inducing transmission experiments on mice, guinea pigs, and rabbits (Helwig and Schmidt 1945). Research in the 1960s, 1970s, and 1980s of the past century developed myocarditis models in mice, rats, pigs, and rabbits as well. In 1963, Kaplan and Craig studied cardiac lesions in rabbit hearts caused by immunization with heterologous heart tissue (Kaplan and Craig 1963). Further studies successively refined immunization strategies and led to the first experimental autoimmune myocarditis models in mice, based on the injection of a mixture of whole mouse heart-specific alpha myosin together with Complete Freund adjuvant (CFA) (Neu et al. 1987). Ethical, logistical, statistical, and economic reasons, the availability of genetically altered mice and an acceptable level of relevance in mirroring human disease mechanisms all explain why mouse and – to a lesser extent – rat models currently dominate autoimmune myocarditis research.

Autoimmunity in Inflammatory Dilated Cardiomyopathy

Inflammatory dilated cardiomyopathy (iDCM) represents an important cause of heart failure in humans and often evolves from progressive myocarditis (Pauschinger et al 1999; Schultheiss et al. 2011). The protozoan Trypanosoma cruzi (Chagas disease) represents a leading cause of myocarditis in South America, but viral infections are the most common triggers in developed countries. The most important cardiotropic viruses are parvovirus B19, HHV-7-viruses, and entero- and adenoviruses (Badorff et al. 1999; Pauschinger et al 1999; Schultheiss et al. 2011). Importantly, infections not only infer direct damage to the heart (Badorff et al. 1999) but may also promote autoimmunity. Autoimmunity develops if the extent of autoreactive T cell activation is sufficient to overcome counter-regulatory tolerance mechanisms in genetically susceptible individuals. Accordingly, many patients with viral myocarditis develop heart-specific autoantibody responses on follow-up (Frustaci et al 2009; Schultheiss et al. 2011). In addition, viral genomes were found in biopsies of patients with dilated cardiomyopathy in the presence or absence of heart-specific autoantibodies. These observational findings suggest that viral infections indeed trigger heart-specific autoimmunity. The finding that immunosuppressive therapy improves heart function in patients without persistence of viral genomes in heart biopsies (Frustaci et al 2009; Schultheiss et al. 2011) further supports the concept that autoimmunity plays a central role in iDCM.

Strong evidence for autoimmunity in inflammatory heart disease, however, results from mouse and rat models. Mice infected with the human pathogen Coxsackie type B3 (CVB3) virus develop myocarditis. In susceptible strains, inflammation progresses even after clearance of the virus (Smith and Allen 1991; Fairweather et al. 2001) and adoptive transfer of T cells, but not serum from diseased mice transfers myocarditis in Severe Combined Immune Deficient (SCID) mice genetically lacking B and T cells. This observation, together with the fact that depletion of $CD4^+$ T cells markedly reduces disease severity, argues for autoimmunity and suggests that T cells contribute to myocarditis progression (Smith and Allen 1991; Pummerer et al. 1996; Fairweather et al. 2001).

Transgenic Models of Autoimmune Heart Disease

Several transgenic myocarditis models have been developed so far. Epidemiologic data suggest an association between dilated cardiomyopathy and specific HLA alleles, such as HLA D6. Consequently, Bachmaier et al. generated double CD4- and CD8-deficient transgenic humanized

mice expressing the human CD4 molecule and HLA antigen. These mice developed autoimmune myocarditis upon immunization with heart-specific peptides and therefore strongly argue for the autoimmunity hypothesis of inflammatory dilated cardiomyopathy in humans (Bachmaier et al. 1999).

NOD mice, on the other hand, contain many genetic susceptibility loci, which cause spontaneous T cell-dependent autoimmune diabetes. Replacement of a specific allele of the MHC class II complex (I-Ag7) with the human DQ8 allele ($DQ8^+$NOD mice) led to protection from NOD autoimmune diabetes but gave rise to spontaneous myocarditis, autoantibodies, and heart failure instead (Lv et al. 2011). Myocarditis in these mice, however, did not result from cardiac autoantibodies, but was clearly the result of autoimmune $CD4^+$ T cell responses. Mechanistically, a defect in thymic negative selection (i.e., the deletion of autoreactive T cells during their development) was underlying autoreactive T cell expansion and cardiac inflammation in the $DQ8^+$NOD mice.

Another recently developed model is based on the generation of mouse heart myosin epitope-specific, T cell receptor (TCR) transgenic mice on a BALB/c background. These mice develop spontaneous myocarditis and die from heart failure (Nindl et al. 2012).

CD4 T cells are critical for Experimental Autoimmune Myocarditis in most mouse strains. In humans, however, viral infections are the most common triggers of postinflammatory cardiomyopathy, at least in developed countries. From this point of view, CD8 T cells cannot be excluded as disease-promoting T cell subsets in the human system. Accordingly, a transgenic mouse model had been developed, which allows us to specifically address the immunopathogenesis of CD8 T cell-mediated cytotoxicity in the absence of viral infections (Grabie et al. 2003). These mice express ovalbumin peptide specifically on cardiac myocytes and develop severe CD8-mediated myocarditis upon OVA immunization. Taking advantage of these mice, as well as of the experimental autoimmune myocarditis model, Tarrio et al. described on the genetic level the potentially important role of PD-1 in myocarditis development. PD-1, a member of the CD28 family of immune regulatory molecules expressed on activated T cells, interacts with its ligands, PD-L1/B7-H1 and PD-L2/B7-DC, on other cells and, thus, delivers inhibitory signals to the T cell. The absence of PD-1 signaling resulted in enhanced cardiac inflammation, mainly due to less limited expansion of autoreactive CD8 and CD4 T cells (Tarrio et al. 2012).

Given the complexity and the myriads of potential inflammatory triggers of heart-specific autoimmune responses, further transgenic models of myocarditis will be developed in the future. Nevertheless, transgenic mice are a quite artificial system. They are helpful to answer specific mechanistic questions, but their relevance in mirroring the transition from infection to autoimmunity remains questionable. One of the most innovative strategies to study mechanisms of human autoimmunity in vivo will certainly include the reconstitution of complete immunodeficient mice (such as the $Rag2^{-/-}gammac^{-/-}$ mice) with a selected complete adaptive human immune system.

Experimental Autoimmune Myocarditis (EAM)

Immunization models offer the advantage to study autoimmune disease mechanisms in vivo without using infective agents. The concept is based on the idea that activation of antigen-presenting cells (APC), taking up and processing self-antigen, is a prerequisite for priming of autoimmune T cell responses. In the absence of appropriate APC activation, however, autoreactive T cell expansion is neither sustainable nor capable to overcome immuno-tolerance in non-transgenic mice and rats. Accordingly, delivery of self-antigen by itself is not sufficient to induce an autoimmune disease phenotype in naïve mice and rats. Therefore, autoantigen has to be applied together with a nonspecific strong adjuvant, such as Complete Freund adjuvant (CFA), containing heat-killed *M. tuberculosis* (Mtb^{hk}) as an active component,

for example. Whole alpha-myosin heavy-chain protein is heart specific and represents the best characterized self-protein for autoimmune myocarditis induction in mice and rats (Neu et al. 1987; Kodama et al. 1990). Unfortunately, the isolation of heart myosin from mice is a cumbersome and time-consuming procedure. Furthermore, whole alpha myosin exerts toxic effects in cell cultures limiting its use in T cell proliferation assays and related immunological in vitro studies. These drawbacks of whole heart myosin immunization can be overcome by using commercially available cross-reactive porcine heart myosin for rat immunization or by using short synthetic peptides representing the most self-pathogenic epitope of either mouse or rat heart myosin (Wegmann et al. 1994; Pummerer et al. 1996) or recombinant mouse cardiac troponin I (Kaya et al. 2008).

Not all mouse and rat strains are equally susceptible to EAM induction. The model works best with female Lewis rats (Kodama et al. 1990). In mice, highest disease scores are observed in A/J, A.CA, and A.SW strains. BALB/c mice are only moderately susceptible, but using specific alpha-myosin peptides rather than whole mouse heart myosin significantly increases disease severity. C57BL/6 and C57BL10 mice are completely resistant (Neu et al. 1987).

Despite highly reproducible and severe inflammation scores in A/J mice after alpha-myosin/CFA immunization, BALB/c mice and myosin peptides are usually preferred as self-antigens. This preference relies on the potential technical difficulties with mouse myosin isolation and purification, the availability of several transgenic models and knockouts on this background, and the facts that synthetic peptides are more practical in cell cultures and proliferation assays. Nevertheless, some of the pathogenic myosin-heavy-chain self-peptides are very hydrophobic and barely soluble in biological fluids and media. Introducing two Arginine residues at the H_2N- and the –COOH terminus of the hydrophobic peptide greatly increases solubility without affecting efficacy and specificity of the immune response. For an immunization of an 8–10-week-old BALB/c mouse, 100–150 μg peptide are required. Hydrophobic peptides can be dissolved in the oily CFA before creating a 1:1 emulsion with phosphate buffered saline. The emulsion is injected subcutaneously into both flanks of the mouse. Mice are boosted after 7 days and first cardiac infiltrates are visible as early as 14 days after the first immunization. Intraperitoneal injection of pertussis toxin on days 0 and 3 additionally enhances myocarditis incidence. Depending on the goal of the experiment, mice will be sacrificed at day 21 (peak of myocarditis) or between days 40 and 60 (if inflammatory dilated cardiomyopathy is the desired phenotype). Of note, disease incidence is rarely >80 % with BALB/c mice. Several nonspecific factors, such as commercial source of the mice, housing conditions, food, and experience of the person doing the immunization, influence disease severity and incidence. This must be taken into account while planning experiments, and performing pilot studies prior to start expensive and time-consuming projects is strongly recommended.

Lewis rat immunization follows a similar protocol. Instead of the standard CFA, however, reliable myocarditis induction requires the use of supplemented (i.e., containing 10 mg/ml heat-killed *Mycobacterium tuberculosis* strain H37Ra) adjuvant. A great advantage of the Lewis rats is the fact that disease incidence and severity are usually reliably high (>90 %). The use of strong adjuvant, however, shifts the histological phenotype to a picture similar to human giant cell myocarditis (Kodama et al. 1990; Wegmann et al. 1994).

Using short self-peptides, EAM can also be induced in BALB/c mice by injection of in vitro-activated α-myosin-peptide-loaded dendritic cells (Eriksson et al. 2003). Dendritic cells are sentinels of the immune system scavenging foreign pathogens as well as cell debris and necrotic tissues. Nonspecific activation of dendritic cells through Toll-like receptors (TLR) is critical for autoreactive T cell priming and can be achieved in vitro with a short (no longer than 2 h!) exposition to 1 μg/ml of lipopolysaccharide (LPS) or other TLR stimulants. Once activated, CD40-ligand-expressing autoreactive T cells

further increase the priming efficacy of the dendritic cells by a positive feedback loop that promotes production of proinflammatory cytokines and survival of activated dendritic cells via CD40-CD40L interaction. Disease induction by vaccination with self-antigen-loaded dendritic cells offers theoretically alternative approach to study APC-effector-cell interactions and priming mechanisms of autoimmune T cells in vivo in the absence of adjuvant. The concept that innate activation of self-antigen-loaded dendritic cells is sufficient to induce autoreactive T cells does not exclude a role for antigenic mimicry or failure of self-tolerance mechanisms in putting the heart at risk for autoimmune attack; it is conceivable that an immune system that was formerly exposed to any microorganisms mimicking self-antigen is more susceptible to boost autoreactive T cells after a second hit releasing self-antigen in the presence of a strong inflammatory response. Clinical and experimental observations strongly support this concept: it explains heart-specific autoimmunity after noninfectious tissue damage, such as cardiac surgery or myocardial infarction (Maisel et al. 1998; Rose 2001). Any tissue damage results in uptake of self-antigens by dendritic cells. If such self-antigen-loaded dendritic cells become activated, they might initiate autoimmune responses depending on the genetic susceptibility of the host.

Adoptive transfer of heart-specific T cells offers another approach to induce autoimmune myocarditis in both rats and mice. Unfortunately, injection of in vitro-restimulated $CD4^+$ T cells isolated from spleens/lymph nodes of mice with EAM results in mild myocarditis in SCID mice or lipopolysaccharide (LPS)-pretreated wild-type animals only (Smith and Allen 1991; Eriksson et al. 2003). The efficacy of adoptive transfer, however, can be greatly improved if heart-specific $CD4^+$ T cells are either isolated from T cell receptor (TCR) transgenic mice (Nindl et al. 2012) or specifically enriched over several cycles of in vitro restimulation/expansion (Valaperti et al. 2008).

It must be emphasized, however, that all immunization strategies are to a certain extent artificial and there is no model, which mirrors exactly the complexity of natural autoimmune disease development in an organism.

Analysis of Cardiac Infiltrates

At the peak of disease, inflammatory infiltrates mainly consist of myelo-derived mononuclear cells, neutrophils, and some T cells. Giant cells are occasionally observed in A/J mice and on a regular basis in Lewis rats, suggesting that the latter two models somehow mirror human giant cell myocarditis. To grade disease severity at this time point, we recommend to follow a well-established semiquantitative scoring system, which allows comparisons between different studies (Neu et al. 1987; Smith and Allen 1991; Wegmann et al. 1994). Importantly, EAM is a patchy disease. From this point of view, we recommend to cut several step sections throughout the heart. Standard hematoxylin-eosin staining is sufficient for analysis. To enhance reproducibility and specificity, mainly in the presence of mild inflammation, two independent and blinded scientists (experienced pathologists preferred) are required for scoring.

Alternatively, inflammatory cells can be quantified with Fluorescence Activated Cell Sorting (FACS) analysis. This technique offers the advantage to reliably specify different cell subsets but is technically difficult to establish and requires profound experience (Afanasyeva et al. 2004; Kania et al. 2008). Nevertheless, flow cytometry and cell sorting allowed a better characterization of heart-infiltrating cells and helped to elucidate their role in pathological remodeling and disease progression (Kania et al. 2008; Cihakova et al. 2008; Barin et al. 2012). FACS analysis confirmed that the majority of heart-infiltrating cells express a monocyte/macrophage phenotype. A substantial proportion of these monocyte-like cells, however, express a pattern of surface markers, which labels them as immature myelo-derived precursors with the capacity to differentiate either in mature monocytes/macrophages into granulocytes or TGF-β dependently into fibroblasts (Kania et al. 2008;

Kania et al. 2009; Blyszczuk et al. 2009). Thus, the major cell subset among cardiac infiltrates not only provides a precursor pool for inflammation-promoting macrophages (Kania et al. 2009; Barin et al. 2012) but also represents the cellular source of subsequent tissue fibrosis (Kania et al. 2009).

Models of Postinflammatory Dilated Cardiomyopathy

Inflammatory dilated cardiomyopathy (iDCM) represents the end-stage heart failure phenotype of progressive myocarditis in humans (Schultheiss et al. 2011). The EAM models provided important insights in the pathomechanisms of immune-mediated cardiac inflammation. Nevertheless, the relevance of these models for human inflammatory dilated cardiomyopathy had been questioned for a long time. This was mainly due to the fact that the readout for disease severity is the extent of cardiac infiltrates after 21 days. Because physical findings in animals at the peak of disease are directly related to histological severity, mice with mild or moderate myocarditis are only minimal symptomatic at this time point. In fact, mortality rates are usually <1 % within 3 weeks after the first immunization. Only A/J mice and Lewis rats develop consistently high disease severity with signs of overt heart failure. Accordingly, pericardial and pleural effusions and ascites can often be seen at autopsy in these animals.

Over the past several years, more long-term follow-up data of mice with EAM became available. In fact, inflammatory infiltrates slowly resolve after day 21 and get successively replaced by fibrotic tissue. In parallel, hearts enlarge and develop a typical end-stage heart failure phenotype with wall thinning, interstitial fibrosis, and excentric hypertrophy. This process of pathological remodeling can be monitored in vivo using echocardiography or cardiac magnetic resonance imaging (MRI) (Eriksson et al. 2003; Blyszczuk et al. 2009; Moon et al. 2012; Nindl et al. 2012). Accordingly, the EAM model also provided valuable insights into disease mechanisms during the late phase of inflammatory heart diseases and allowed conclusions regarding iDCM development in humans (Afanasyeva et al. 2005; Blyszczuk et al. 2009). Thus, it was possible to define novel roles for several key cytokines in iDCM progression, which go beyond their well-known significance in mediating autoreactive T cell responses. The absence of Interleukin-17A (IL-17A), for example, did not prevent acute myocarditis, but almost completely protected from progression to cardiomyopathy by inhibiting pathological remodeling and cardiac fibrosis (Baldeviano et al. 2010). Similar protective effects are observed in anti-TGF-β-treated, myosin/CFA-immunized mice (Kania et al. 2009) or in animals lacking the IL-1 receptor-linked, downstream adaptor molecule MyD88 (Blyszczuk et al. 2009). Both TGF and IL-1 are closely linked to IL-17. In fact, increasing evidence suggests a critical role for IL-17 in chronic progressive inflammatory responses and fibrogenesis that go beyond its role as key cytokine of a distinct helper cell subset. Despite the not yet clarified role for IL-17 in EAM induction, IL-17 therefore appears as promising target for the prevention of heart failure progression.

The optimal time schedule to study mechanisms of iDCM development has not been defined yet. Forty to sixty days after the first immunization are sufficient for most experiments to get micro- and macroscopic evidence of pathological remodeling and reproducible changes in systolic or diastolic echocardiographic parameters, without reaching an ethically problematic level of symptomatic disease burden in mice.

Outlook

EAM models became helpful in vivo tools to study the pathogenesis of human inflammatory heart diseases. On one hand, they illustrate the cooperation between innate and adaptive immunity as the critical event triggering autoimmunity. On the other hand, they help to understand immunological mechanisms underlying disease development and progression to end-stage heart failure. Nevertheless, it is not known exactly yet to what extent these observations can be

transferred to the human system. Additional studies are clearly needed. Meanwhile, however, autoimmune myocarditis models already offer attractive tools to support the development of innovative and novel treatment strategies.

Summary

Several lines of evidence indicate that autoimmune mechanisms contribute to the pathogenesis of iDCM, which represents an end-stage phenotype of progressive, mostly virus-triggered, myocarditis. Several models of myocarditis – mostly on rats and mice – have been developed. Transgenic mice can answer specific questions on potential disease mechanisms. Most insights into the immune mechanisms of iDCM development, however, result from the EAM model in mice and rats. EAM is based on the observation that susceptible animals injected with heart-specific, self-antigen-loaded dendritic cells or immunized with self-antigen together with a strong adjuvant develop cardiac inflammation peaking 21 days after the first immunization. Later on, myocarditis progresses to an end-stage heart failure phenotype, which resembles in many aspects of iDCM in humans. Despite the simple immunization procedure, however, reliable results depend critically on careful protocols, appropriate strain, sex, and age selection, and on careful interpretation of histology or imaging studies. Nevertheless, the EAM models provided important hints to relevant mechanisms and potential novel treatment strategies in human iDCM.

Cross-References

- ▶ Autoimmune Myocarditis and Pericarditis
- ▶ CD40
- ▶ Epigenetics in Autoimmunity
- ▶ Resolution of Inflammation
- ▶ T cell Memory
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver
- ▶ Viral Myocarditis

References

Afanasyeva M, Georgakopoulos D, Belardi DF, Ramsundar AC, Barin JG, Kass DA, Rose NR. Quantitative analysis of myocardial inflammation by flow cytometry in murine autoimmune myocarditis: correlation with cardiac function. Am J Pathol. 2004;164:807–15.

Afanasyeva M, Georgakopoulos D, Belardi DF, Bedja D, Fairweather D, Wang Y, Kaya Z, Gabrielson KL, Rodriguez ER, Caturegli P, Kass DA, Rose NR. Impaired up-regulation of CD25 on $CD4^+$ T cells in IFN-γ knockout mice is associated with progression of myocarditis to heart failure. Proc Natl Acad Sci USA. 2005;102:180–5.

Bachmaier K, Neu N, Yeung RS, Mak TW, Liu P, Penninger JM. Generation of humanized mice susceptible to peptide-induced inflammatory heart disease. Circulation. 1999;99:1885–91.

Badorff C, Lee GH, Lamphear BJ, Martone ME, Campbell KP, Rhoads RE, Knowlton KU. Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. Nat Med. 1999;5(3):320–6.

Baldeviano CG, Barin JG, Talor MV, Srinivasan S, Bedja D, Zheng D, Gabrielson K, Iwakura Y, Rose NR, Cihakova D. Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. Circ Res. 2010;106:1646–55.

Barin JG, Baldeviano GC, Talor MV, Wu L, Ong S, Quader F, Chen P, Zheng D, Caturegli P, Rose NR, Cihákova D. Macrophages participate in IL-17-mediated inflammation. Eur J Immunol. 2012;42(3):726–36.

Blyszczuk P, Kania G, Dieterle T, Marty RR, Valaperti A, Bertonneche C, Pedrazzini T, Berger CT, Dirnhofer S, Matter CM, Penninger JM, Lüscher TF, Eriksson U. MyD88/IL-1 signaling controls cardiac fibrosis and heart failure progression in inflammatory dilated cardiomyopathy. Circ Res. 2009;105:912–20.

Cihakova D, Barin JG, Afanasyeva M, Kimura M, Fairweather D, Berg M, Talor MV, Baldeviano GC, Frisancho S, Gabrielson K, Bedja D, Rose NR. Interleukin-13 protects against experimental autoimmune myocarditis by regulating macrophage differentiation. Am J Pathol. 2008;172:1195–208.

Eriksson U, Ricci R, Hunziker L, Kurrer MO, Oudit GY, Watts TH, Sonderegger I, Bachmaier K, Kopf M, Penninger JM. Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. Nat Med. 2003;9:1484–90.

Fairweather D, Kaya Z, Shellam GR, Lawson CM, Rose NR. From infection to autoimmunity. J Autoimmun. 2001;16:175–86.

Frustaci A, Russo MA, Chimenti C. Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: the TIMIC study. Eur Heart J. 2009;30(16):1995–2002.

Grabie N, Delfs MW, Westrich JR, Love VA, Stavrakis G, Ahmad F, Seidman CE, Seidman JG, Lichtman AH. IL-12 is required for differentiation of pathogenic $CD8^+$ T cell effectors that cause myocarditis. J Clin Invest. 2003;111:671–80.

Helwig FC, Schmidt CH. A filter-passing agent producing interstitial myocarditis in anthropoid apes and small animals. Science. 1945;102(2637):31–3.

Kania G, Blyszczuk P, Valaperti A, Dieterle T, Leimenstoll B, Dirnhofer S, Zulewski H, Eriksson U. Prominin-1^+/$CD133^+$ bone marrow-derived heart-resident cells suppress experimental autoimmune myocarditis. Cardiovasc Res. 2008;80:236–45.

Kania G, Blyszczuk P, Stein MS, Valaperti A, Germano D, Dirnhofer S, Hunziker L, Matter CM, Eriksson U. Heart-infiltrating prominin-1^+/$CD133^+$ progenitor cells represent the cellular source of TGF-β-mediated cardiac fibrosis in experimental autoimmune myocarditis. Circ Res. 2009;105:462–70.

Kaplan MH, Craig JM. Immunologic studies of heart tissue: VI. Cardiac lesions in rabbits associated with autoantibodies to heart induced by immunization with heterologous heart. J Immunol. 1963;90:725–33.

Kaya Z, Göser S, Buss SJ, Leuschner F, Ottl R, Li J, Völkers M, Zittrich S, Pfitzer G, Rose NR, Katus HA. Identification of cardiac troponin I sequence motifs leading to heart failure by induction of myocardial inflammation and fibrosis. Circulation. 2008;118:2063–72.

Kodama M, Matsumoto Y, Fujiwara M, Masani F, Izumi T, Shibata A. A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction. Clin Immunol Immunopathol. 1990;57:250–62.

Lv H, Havari E, Pinto S, Gottumukkala R, Cornivelli L, Raddassi K, Matsui T, Rosenzweig A, Bronson RT, Smith R, Fletcher AL, Turley SJ, Wucherpfennig K, Kyewski B, Lipes MA. Impaired thymic tolerance to alpha- myosin directs autoimmunity to the heart in mice and humans. J Clin Invest. 2011;121:1561–73.

Maisel A, Cesario D, Baird S, Rehman J, Haghighi P, Carter S. Experimental autoimmune myocarditis produced by adoptive transfer of splenocytes after myocardial infarction. Circ Res. 1998;82:458–63.

Moon H, Park HE, Kang J, Lee H, Cheong C, Lim YT, Ihm SH, Seung KB, Jaffer FA, Narula J, Chang K, Hong KS. Noninvasive assessment of myocardial inflammation by cardiovascular magnetic resonance in a rat model of experimental autoimmune myocarditis. Circulation. 2012;125:2603–12.

Neu N, Rose NR, Beisel KW, Herskowitz A, Gurri-Glass G, Craig SW. Cardiac myosin induces myocarditis in genetically predisposed mice. J Immunol. 1987;139:3630–6.

Nindl V, Maier R, Ratering D, De Giuli R, Züst R, Thiel V, Scandella E, Di Padova F, Kopf M, Rudin M, Rülicke T, Ludewig B. Cooperation of Th1 and Th17 cells determines transition from autoimmune myocarditis to dilated cardiomyopathy. Eur J Immunol. 2012;42:2311–21.

Pauschinger M, Doerner A, Kuehl U, Schwimmbeck PL, Poller W, Kandolf R, Schultheiss HP. Enteroviral RNA replication in the myocardium of patients with left ventricular dysfunction and clinically suspected myocarditis. Circulation. 1999;99:889–95.

Pummerer CL, Luze K, Grassl G, Bachmaier K, Offner F, Burrell SK, Lenz DM, Zamborelli TJ, Penninger JM, Neu N. Identification of cardiac myosin peptides capable of inducing autoimmune myocarditis in BALB/c mice. J Clin Invest. 1996;97:2057–62.

Rose NR. Infection, mimics, and autoimmune disease. J Clin Invest. 2001;107:943–4.

Schultheiss HP, Kühl U, Cooper LT. The management of myocarditis. Eur Heart J. 2011;32:2616–25.

Smith SC, Allen PM. Myosin-induced acute myocarditis is a T cell-mediated disease. J Immunol. 1991;147:2141–7.

Tarrio ML, Grabie N, Bu DX, Sharpe AH, Lichtman AH. PD-1 protects against inflammation and myocyte damage in T cell-mediated myocarditis. J Immunol. 2012;188:4876–84.

Valaperti A, Marty RR, Kania G, Germano D, Mauermann N, Dirnhofer S, Leimenstoll B, Blyszczuk P, Dong C, Mueller C, Hunziker L, Eriksson U. $CD11b^+$ monocyte abrogate Th17 $CD4^+$ T cell-mediated experimental autoimmune myocarditis. J Immunol. 2008;180:2686–95.

Wegmann KW, Zhao W, Griffin AC, Hickey WF. Identification of myocarditogenic peptides derived from cardiac myosin capable of inducing experimental allergic myocarditis in the Lewis rat. J Immunol. 1994;153:892–9.

Autoimmune Hepatitis

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Synonyms

Autoimmune chronic active hepatitis; Autoimmune hepatitis (AIH); Lupoid hepatitis

Definition

Autoimmune hepatitis (AIH) is an inflammatory liver disorder that affects mainly females and characterized by elevated transaminase

levels, autoantibody positivity, immunoglobulin G (IgG) elevation, and the histological picture of interface hepatitis (Krawitt 2006; Mieli-Vergani and Vergani 2011a). Immune reactions against liver antigens are likely to be the major pathogenic mechanism (Vergani and Mieli-Vergani 2012).

Clinical and Systemic Manifestations

Historical Background – The first full description of AIH was given by Waldeström in 1950 when he reported six patients, five female, with hepatitis and marked elevation of gamma globulins in whom ACTH, administered to investigate concomitant amenorrhea, resulted in clinical and biochemical improvement. In 1956, Mackay reported the presence of lupus erythematosus (LE) cells in patients with a similar clinical picture and proposed the term "lupoid hepatitis." Later, when it became clear that liver disease is rare in systemic lupus erythematosus and that LE cells reflect the presence of antinuclear antibody, Mackay suggested the name of "autoimmune hepatitis," which was universally accepted in the 1990s (Vergani and Mieli-Vergani 2007).

The diagnosis of AIH is based on positive findings and exclusion of other causes of liver disease. Diagnostic criteria have been defined and revised by the International Autoimmune Hepatitis Group (Johnson and McFarlane 1993; Alvarez et al. 1999; Hennes et al. 2008). Positive criteria are elevation of transaminase and IgG levels, presence of diagnostic autoantibodies, and interface hepatitis on liver biopsy (i.e., a dense portal tract inflammatory infiltrate composed of lymphocytes, monocytes, and plasma cells, which crosses the limiting plate and invades the surrounding parenchyma). The liver biopsy is important not only for diagnostic purposes but also for the evaluation of the severity of liver injury, as the degree of biochemical and immunological abnormalities does not reflect the extent of the histological damage. Other hepatic disorders, such as viral hepatitis B and C, Wilson disease, and alcohol- or drug-induced liver disease, which may share clinical and biochemical features of AIH, must be excluded.

AIH Subtypes – AIH is divided in two main forms according to the type of autoantibody present: smooth muscle antibody (SMA) and/or antinuclear antibody (ANA) define AIH type 1, while liver/kidney microsomal type 1 antibody (anti-LKM-1) and liver cytosol type 1 antibody (anti-LC-1) define AIH type 2 (Vergani et al. 2004). As anti-LKM-1 positive disease is infrequent in adults, the distinction in type 1 and type 2 AIH is particularly relevant in pediatric age, where anti-LKM-1 positive AIH represents one-third of all cases. Type 1 and type 2 AIH have a similar clinical course, though anti-LKM-1 positive children present at a younger age, more often with an acute onset, including fulminant hepatitis, and have associated IgA deficiency (Mieli-Vergani and Vergani 2011b).

Other autoantibodies that support the diagnosis of AIH are anti-soluble liver antigen (anti-SLA) and perinuclear antineutrophil cytoplasmic antibodies (pANCA). The former is highly specific, though not very sensitive, for the diagnosis of AIH and can be present in the absence of conventional diagnostic autoantibodies. The latter is frequent in AIH/sclerosing cholangitis overlap syndrome and in primary sclerosing cholangitis (Vergani and Mieli-Vergani 2006).

Prevalence and Systemic Manifestations – AIH affects 2–20/100,000 people and is more frequent in females, who represent 75 % of the cases (Mieli-Vergani and Vergani 2011a). Some 40 % of patients have a family history of autoimmune disease, and at least one-fifth of the patients suffer from other autoimmune disorders either at diagnosis or during follow-up, including thyroiditis, ulcerative colitis, insulin-dependent diabetes, systemic lupus erythematosus, vitiligo, nephrotic syndrome, hypoparathyroidism, and Addison disease. The latter two endocrinological disorders are observed mainly in anti-LKM-1 positive patients or in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), an autosomal recessive monogenic disorder with a variable phenotype that includes AIH in about 20 % of cases (Mieli-Vergani and Vergani 2011b).

Presentation – There are three main patterns of disease presentation: (1) an acute onset, characterized by nonspecific symptoms of malaise, nausea/vomiting, anorexia, and abdominal pain, followed by jaundice, dark urine, and pale stools (30–40 % of cases); (2) an insidious onset, with an illness characterized by progressive fatigue, relapsing jaundice, headache, anorexia, amenorrhea, and weight loss (50–60 % of cases); and (3) a presentation with complications of portal hypertension, such as hematemesis from esophageal varices, bleeding diathesis, chronic diarrhea, weight loss, and vomiting (5–10 % of cases). Since the mode of presentation is so variable, AIH should be suspected in all patients presenting with symptoms and signs of prolonged or severe liver disease. Occasional patients, however, are asymptomatic and diagnosed after incidental discovery of abnormal liver function tests.

Treatment

AIH responds well to immunosuppressive treatment, which should be instituted immediately after diagnosis (Vergani and Mieli-Vergani 2011).

The aim of treatment is to achieve early complete remission to prevent disease progression and to maintain remission long term on the lowest possible drug dose (Manns et al. 2010). Remission is obtained in about 80 % of cases, whatever the disease presentation, apart from those rare patients with fulminant hepatitis, in whom disease is usually too advanced for medical treatment to be effective and who usually require liver transplantation.

Standard Treatment – A combination of predniso(lo)ne and azathioprine is the basis of treatment for AIH. The old suggestion of waiting for 6 months before starting treatment is now obsolete, as early treatment has been shown to halt disease progression, which can be otherwise very rapid (Manns et al. 2010). Most AIH patients who respond to immunosuppressive treatment have an excellent prognosis and lead a normal life on low-dose medication.

The 2010 American Association for the Study of Liver Diseases (AASLD) practice guidelines (Manns et al. 2010) recommend starting treatment in adult patients with either a combination of predniso(lo)ne (30 mg) and azathioprine (1–2 mg/kg) daily or with prednisone monotherapy at a starting dose of 40–60 mg daily. For children up to 18 years of age, a dose of 1–2 mg/kg predniso(lo)ne (maximum daily dose 60 mg) is recommended in combination with azathioprine (1–2 mg/kg/daily) or 6-mercaptopurine (1.5 mg/kg). However, as azathioprine can be hepatotoxic, especially in the presence of jaundice, it is advisable to add it in icteric patients only when partial disease control is achieved and jaundice has subsided. The most common side effects of steroids are cushingoid changes; less common adverse events include osteoporosis, vertebral collapse, diabetes, cataract, hypertension, and psychosis. However, only about 13 % of patients necessitate dose reduction or premature steroid withdrawal because of side effects (Vergani and Mieli-Vergani 2007). Side effects of azathioprine, including cholestatic hepatitis, venoocclusive disease, pancreatitis, nausea and vomiting, rash, and bone marrow suppression, affect less than 10 % of patients and usually settle after drug withdrawal. As myelosuppression during treatment has been reported only in patients with near-zero erythrocyte concentrations of thiopurine methyltransferase activity, its measurement is only warranted in the presence of pretreatment or intra-treatment cytopenia or when higher than conventional doses of azathioprine are needed to maintain remission (Mieli-Vergani and Vergani 2011a).

Complete remission is defined as disappearance of clinical symptoms, normalization of transaminase and IgG levels, and negativization or reduction to a very low titer of the autoantibodies (Manns et al. 2010; Mieli-Vergani and Vergani 2011a). Histological resolution of inflammation occurs months after biochemical improvement. As AIH responds swiftly to immunosuppressive treatment, lack of improvement on steroids suggests other causes of liver disease.

Relapse, characterized by an increase of transaminase levels, occurs in 40–80 % of patients, often during attempts of treatment withdrawal or as a result of nonadherence. Episodes of relapse usually respond to a temporary increase in the steroid dose. Nonadherence to treatment is a serious problem particularly in young adults and adolescents (Mieli-Vergani and Vergani 2011b).

Most patients, including those with cirrhosis, reach complete remission on treatment. In the presence of significant steroid side effects, remission can be maintained with azathioprine alone at a dose of 2 mg/kg daily. Treatment with both steroids and azathioprine is safe during pregnancy (Mieli-Vergani and Vergani 2011a), as neither drug has been reported to have teratogenic properties in humans. However, pregnant women anxious about the use of azathioprine can be switched to steroid monotherapy.

In asymptomatic or paucisymptomatic adult patients, the benefit of therapy should be weighed against corticosteroid side effects, particularly in postmenopausal women or elderly patients. The decision to treat depends on the severity of inflammation and liver damage.

Children have a more aggressive disease than adults, despite being often paucisymptomatic; hence, treatment should be started promptly in childhood, even if the diagnosis is made incidentally (Czaja et al. 2005).

Most authors recommend at least 3 years of continuous therapy, before considering drug withdrawal (Manns et al. 2010). Attempts to stop treatment should be made only if a liver biopsy shows resolution of the inflammatory changes. During treatment withdrawal the patient must be monitored closely, as relapse may be severe and even fatal.

The risk of developing primary hepatocellular carcinoma (HCC) in AIH is associated with the presence of cirrhosis, akin to other chronic liver diseases; hence, surveillance for HCC is advisable (Manns et al. 2010).

Alternative Treatments – Ciclosporin and tacrolimus have been used as steroid-sparing agents, but whether the use of these toxic and expensive drugs confers any advantage over standard treatment is unclear.

Budesonide, a corticosteroid with a high first pass liver metabolism, has been used to induce remission in AIH with a marginal benefit when compared to conventional corticosteroids (Mieli-Vergani and Vergani 2011a).

Difficult-to-treat cases respond to mycophenolate mofetil at a dose of 20 mg/kg twice daily in association with predniso(lo)ne, although this drug appears more effective in patients intolerant of but not in those unresponsive to azathioprine (Lohse and Mieli-Vergani 2011). For patients who do not respond to or who develop mycophenolate mofetil side effects (headache, diarrhea, nausea, dizziness, hair loss, and neutropenia), calcineurin inhibitors should be considered in combination with predniso(lo)ne (Manns et al. 2010). In particularly difficult to treat patients, anecdotally, the use of the anti-B-cell monoclonal antibody rituximab and of the antitumor necrosis factor alpha monoclonal antibody infliximab has been reported with variable outcomes (Vergani and Mieli-Vergani 2011). An important risk of these biological treatments is the occurrence of severe infections.

Liver Transplantation – Liver transplantation is the treatment of choice for those rare patients who present with fulminant hepatic failure (grade II–IV hepatic encephalopathy) or for the 10–20 % of cases that progress to end-stage liver disease despite immunosuppression. AIH recurrence, characterized by high transaminase levels, positive autoantibodies, interface hepatitis, and/or steroid dependence, occurs in some 20 % of transplanted patients (Liberal et al. 2012). Steroid treatment long term and at a dose higher than that generally used after liver transplantation for other conditions are recommended to try and avoid recurrence.

Future Treatments – Over the past 10 years, it has become apparent that loss of immunoregulation is central to the pathogenesis of AIH (Longhi et al. 2010), as in this condition there is a reduction in number and function of regulatory T cells (Tregs) especially during the active phases of the disease. Current research is focusing on restoring both number and function of Tregs in vitro to reinfuse them in patients in an attempt to halt/cure the disease by reinstating immune tolerance to self.

Cross-References

- ▶ Autoimmune Hepatitis: Pathogenesis, Association with Other Syndromes
- ▶ Animal Models of Autoimmune Hepatitis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Transplantation for Autoimmune Liver Diseases
- ▶ Tregs in the Liver

References

Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol. 1999;31(5):929–938.

Czaja AJ, Bianchi FB, Carpenter HA, Krawitt EL, Lohse AW, Manns MP, et al. Treatment challenges and investigational opportunities in autoimmune hepatitis. [Review]. Hepatology. 2005;41(1):207–215.

Hennes EM, Zeniya M, Czaja AJ, Pares A, Dalekos GN, Krawitt EL, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology. 2008;48(1):169–176.

Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. Hepatology. 1993;18(4):998–1005.

Krawitt EL. Autoimmune hepatitis. N Engl J Med. 2006;354(1):54–66.

Liberal R, Longhi MS, Grant CR, Mieli-Vergani G, Vergani D. Autoimmune hepatitis after liver transplantation. [Research Support, Non-U.S. Govt Review]. Clin Gastroenterol Hepatol. 2012;10(4):346–353.

Lohse AW, Mieli-Vergani G. Autoimmune hepatitis. [Review]. J Hepatol. 2011;55(1):171–182.

Longhi MS, Ma Y, Mieli-Vergani G, Vergani D. Aetiopathogenesis of autoimmune hepatitis. [Review]. J Autoimmun. 2010;34(1):7–14.

Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, et al. Diagnosis and management of autoimmune hepatitis. Hepatology. 2010;51(6):2193–2213.

Mieli-Vergani G, Vergani D. Autoimmune hepatitis. [Review]. Nat Rev Gastroenterol Hepatol. 2011a;8(6):320–329.

Mieli-Vergani G, Vergani D. Autoimmune liver diseases in children - what is different from adulthood? [Review]. Best Pract Res Clin Gastroenterol. 2011b;25(6):783–795.

Vergani D, Alvarez F, Bianchi FB, Cançado EL, Mackay IR, Manns MP, et al. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. J Hepatol. 2004;41(4):677–683.

Vergani D, Mieli-Vergani G. Hepatic diseases – Sclerosing cholangitis. In: Mackay IR, Rose NR, editors. The autoimmune diseases. 4th ed. Amsterdam: Elsevier Academic Press; 2006. p. 767–777.

Vergani D, Mieli-Vergani G. Autoimmune hepatitis. Textbook of hepatology: from basic science to clinical practice. 3rd ed.. Oxford, UK: Blackwell Publishing, 2007. pp. 1089–1101.

Vergani D, Mieli-Vergani G. Pharmacological management of autoimmune hepatitis. [Review]. Expert Opin Pharmacother. 2011;12(4):607–613.

Vergani D, Mieli-Vergani G. Cutting edge issues in autoimmune hepatitis. Clin Rev Allergy Immunol. 2012;42(3):309–321.

Autoimmune Hepatitis: Pathogenesis, Association with Other Syndromes

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Synonyms

Autoimmune hepatitis

Definition

Autoimmune hepatitis (AIH) is a rare inflammatory liver disease that is clinically characterized by elevations of transaminases (ALT > AST), immunoglobulins, and certain autoantibodies (AAB) as well as exclusion of other relevant causes of hepatitis (e.g., infectious, toxic, vascular, metabolic). Histological hallmarks are the predominantly lymphocytic, mostly plasma cell-rich portal infiltrates with interface hepatitis, although histology alone is never used as diagnosis. A successful treatment with steroids can strengthen the diagnosis of AIH.

Immunopathogenesis

The presence of liver-specific autoantibodies against cytochromes (UDP-glucuronosyltransferases (UGTs) and formiminotransferase cyclodeaminase (FTCD)), the elevation of immunoglobulins, and the dense lymphocytic portal infiltrates suggested an autoaggressive cellular immune response. This was further supported by successful therapy with immunosuppressants (Kirk et al. 1980; Manns et al. 2010).

Break of Humoral Tolerance

The appearance of organ-specific (e.g., liver cytosol type 1 (LC1)) as well as non-organ-specific (e.g., antinuclear antibodies (ANA), anti smooth muscle antibodies (SMA), anti-liver kidney microsome (LKM) antibodies, soluble liver antigen/liver pancreas (SLA/LP)) AAB is one of the key features of AIH and is therefore used in diagnosis to distinguish AIH from other inflammatory liver diseases. These AAB also help to divide AIH into at least two subtypes (type I: ANA and/or SMA; type II: LKM and/or LC1; putative type III: SLA) (Strassburg and Manns 2002).

The discovery that hepatocytes from AIH patients were covered with immunoglobulins and were susceptible to antibody-mediated cytotoxicity first suggested that these AAB might have some pathophysiological relevance. Furthermore in AIH type II, the main target antigen, cytochrome P450 2D6 (CYP2D6), is recognized by AAB (LKM) as well as by T cell receptors (TCR). CYP2D6 is expressed at the surface of hepatocytes and thus directly accessible to AAB. In contrast, the epitopes of ANA, SLA, and SMA, AAB are not only expressed in the liver and are not present on the surface of hepatocytes. These autoantigens are probably accessible to the immune system after apoptotic cell death (Wachter et al. 1990; Longhi et al. 2010b; Lleo et al. 2011).

From a clinical point of view, various AAB are associated with markers of biochemical and histological disease activity (Longhi et al. 2010b), and anti-SLA positivity defines usually a more severe disease course (Czaja 2010). Furthermore, plasma cells, antibody-producing B cells, are mostly found in the expanded portal tract in AIH and may indicate an AAB production in the liver itself, although the specificity of the plasma cells is not known (Oo et al. 2010).

On the other hand, 10–20 % of AIH patients have no relevant AAB titers at diagnosis or of other specificities than the ones routinely tested (Oo et al. 2010). Furthermore, AAB are commonly found in other chronic liver diseases too, e.g., hepatitis C (Longhi et al. 2010b; Boberg et al. 2011). Most of the antibodies are not liver disease specific, and most target antigens are not specifically expressed in the liver. Why ubiquitously expressed antigens become a target of a liver-specific autoimmune response remains difficult to explain. The altered-self hypothesis postulates that common antigens are altered in the liver by substances generated during the detoxification process in the liver. It is remarkable in this context that many of the AAB recognize liver-specific antigens related to the hepatic detoxification machinery (cytochrome P450s, UGTs). T cells specific for these chemically altered neoantigens cannot be negatively selected in the thymus explaining the fulminant clinical presentation of the disease that is occasionally observed in pediatric patients.

Although these AAB are used to diagnose AIH, it is not clear whether they are involved in the pathophysiology leading to chronic immune-mediated destruction of hepatocytes. In this context, it is interesting to note that serum transfer did not lead to hepatitis in an animal model of AIH (Hardtke-Wolenski et al. 2013). On the other hand, experiments in other tissue-specific autoimmune diseases like type 1 diabetes have suggested a role of autoantibodies especially in the initiation of autoimmunity by enhanced antigen uptake and presentation (Greeley et al. 2002; Hardtke-Wolenski et al. 2011).

Break of Self-Tolerance

The peripheral blood of AIH patients contains mostly autoreactive T helper cells while the liver contains mostly autoreactive cytotoxic (► Cytotoxic T Lymphocytes) and γδ T cells.

Clonal analysis has revealed the presence of both CD4 and CD8 T cells reactive against various autoantigens in AIH (e.g., asialoglycoprotein receptor (ASGPR), alcohol dehydrogenase, CYP2D6 and SLA) (Mix et al. 2008). Further investigations have shown that the polyclonal T cell reactivity and T cell-derived cytokine production correlated with biochemical and histological disease activity. Autoreactive T cell clones could also stimulate AAB production by anti-ASGPR-specific B cells in vitro (Longhi et al. 2010b; Lleo et al. 2011). Altogether, numerous effector cell lineages of the adaptive immune system seem to be involved in the autoreactive liver damage in AIH. The reactivity of CD4, CD8 T cells, and B cells against the same or adjacent epitopes suggests a functional interplay of the adaptive immune system in AIH (Longhi et al. 2010b; Lleo et al. 2011).

In recent years, the role of regulatory T cells (Treg; ▶ Tregs in the Liver) in AIH has attracted more attention. Tregs, which constitute about 5 % of the peripheral CD4 T cell pool, play a major role in controlling immune activation in a cell-contact dependent as well as in a cell-contact independent manner by producing anti-inflammatory cytokines (Sakaguchi et al. 2010).

Several reports described a quantitative as well as a functional impairment of Treg in the peripheral blood of pediatric AIH patients that in part could be restored under therapy and during disease remission (Longhi et al. 2010b).

A major obstacle in the analysis of Tregs in human is the broad phenotypic similarity of activated conventional effector T cells (Teff) and Treg and the lack of markers expressed exclusively by Tregs. More markers have been used to characterize Tregs in the last years. These include FOXP3 expression, reduced CD127 expression, and DNA demethylation of a preserved promoter region of the FOXP3 gene locus in addition to CD4+CD25+ (Sakaguchi et al. 2010). As a consequence, the contamination of activated Teff in the population of analyzed Treg is variable and depends on the marker panel used to define Tregs. Using a more extended panel to characterize Tregs, a recent study showed that AIH patients did not display a deficiency in Treg number or function (Peiseler et al. 2012). Interestingly, IPEX (immune dysfunction, polyendocrinopathy, enteropathy, X-linked) patients, who lack completely Tregs or have a profound defect of Treg function, develop numerous autoimmune diseases, but rarely AIH (Gambineri et al. 2008). Thus, a general defect in Tregs is not the likely cause for AIH development.

Other mechanisms have been proposed to explain impaired immunoregulation in AIH. Downregulation of Tim3 expression on Teff cells might decrease their sensitivity to Treg regulation, while reduced Galectin-9 on Tregs might decrease their suppressive capacity (Liberal et al. 2012).

A defect in innate immunity has also been suggested to play an important role in the pathogenesis of AIH. As Monocytes from AIH patients are more activated than those from healthy individuals (Longhi et al. 2010a). In addition, the number and function of NKT cells in AIH patients is decreased, while the number of γδ T cells in the peripheral blood of AIH patients is increased in comparison to control individuals (Ferri et al. 2010; Liberal et al. 2011).

Although both innate and adaptive immune cells seem to be involved in the autoreactive liver inflammation, the events initiating this harmful interplay are not completely understood.

Genetic Associations

Genetic association studies are powerful tools to identify genes or molecules that have a potential pathophysiological contribution in human diseases. As in various other autoimmune diseases, the strongest genetic association in AIH affects genes that encode molecules related to T cell recognition and activation.

MHC Complex

Class II MHC Molecules

The strongest genetic predisposition in AIH has been linked to major histocompatibility complex (MHC) class II genes. These are located on the short arm of chromosome 6, more specifically in

Autoimmune Hepatitis: Pathogenesis, Association with Other Syndromes, Table 1 Genetic association of the HLA-DR haplotypes with AIH type I

	HLA-DR haplotype		
	Predisposition		Protection
Ethnic group	*Major association*	*Secondary association*	
White northern European background	DRB1 *0301 DRB1*0401 DRB3 *0101		
North Americans		DRB1 *13	
Japanese	DRB1*0405		
Latin America	DRB1 *1301	DRB1 *03	DRB1*1302
Mexicans	DRB1 *0404		

the human leukocyte antigen (HLA)-DR locus (Liberal et al. 2011; Oliveira et al. 2011). The class II MHC molecules present peptide sequences (epitopes) to T helper cells.

The genetic predisposition is different in AIH type I and II, most probably reflecting the different antigens, against which the autoimmune attack is targeted. While HLA-DRB1*0701 and/or DRB1*0301 confer the strongest risk for AIH type II, the set of predisposing and protective HLA haplotypes is shaped by the ethnic background in AIH type I (Table 1) (Liberal et al. 2011; Oliveira et al. 2011).

The association of AIH with HLA-DR3 (DRB1 *0301) and DR4 (DRB1 *0401) is considered strong enough, to be used as a criteria in the diagnosis of AIH type I in Caucasians in the diagnostic AIH scoring system (Liberal, Longhi et al. 2011). Both molecules share a homologous amino acid sequence at position 67–72 (LLEQKR). The haplotypes DRB1 *0404 and DRB1 *0405, predisposing to AIH in Japan as well as in Middle and South America (Table 1), exhibit a similar sequence at the same position (LLEQRR), implying a putative role for presentation of similar autoantigens in AIH. In other studies, particular sequences of HLA alleles (e.g., a histidine at position 13 of the DRB1 allele in Japan or a valine/glycine dimorphism at position 86 of the DR-beta polypeptide in South America) are associated with AIH (Liberal et al. 2011).

As mentioned above, the clinical course of AIH is variable and can depend on the types of AAB detected (e.g., more severe course with SLA positivity) (Czaja 2010). The HLA haplotype is also associated with the course and prognosis in AIH type II, suggesting that the course of the disease is influenced by the presented respectively recognized target antigen. While HLA-DR3 (DRB1*0301) is associated with anti-LKM1 and LC1 positivity, HLA-DR7 (DRB1*0701) is associated only with anti-LKM1 positivity, a more restricted anti-LKM1 immunoglobulin repertoire and even with a more severe AIH course (Liberal et al. 2011).

Non-MHC Genes

CTLA-4/CD152

CTLA-4 is a membrane-bound molecule expressed by T cells that act as a negative feedback loop of the T cell activation cascade by competing with CD28, a costimulatory molecule that binds to the same ligands as CTLA-4 (B7-1/CD80 and B7-2/CD86) expressed on antigen-presenting cells. Due to its regulatory role in T cell activation, CTLA-4 is thought to play a critical role in the maintenance of peripheral tolerance (► CTLA-4; ► B7 and CD28 Families). Although associations of polymorphisms of CTLA-4 have been reported in several populations (e.g., an A/G polymorphism at position 49 in exon 1 in Caucasian), they could not be confirmed in all populations (e.g., in Latin Americans or Japanese). Nonetheless, polymorphisms of the CTLA-4 gene have been associated with other autoimmune diseases like diabetes type 1 and multiple sclerosis (Tang, Zhou et al. 2012). The influence of the genetic variability of the CTLA-4 gene does not just affect negative costimulation on effector T cells. CTLA-4 is also constitutively expressed on Tregs and has important regulatory functions (Shevach 2009).

Fas Gene

The Fas receptor is thought to be involved in the maintenance of the peripheral tolerance by induction of receptor-mediated programmed cell death (apoptosis) of autoreactive T cells in the periphery (► Fas/Fas Ligand). Although a genetic link

of Fas polymorphisms with AIH and development of liver cirrhosis has been reported (Tang et al. 2012), there is no clear evidence that this molecule is a susceptibility gene for AIH.

Tumor Necrosis Factor (TNF)

The TNFA*2 gene is another example, in which a genetic link has been reported in some populations (e.g., European and North American Caucasian) but could not be confirmed in others (e.g., Latin American) (Tang et al. 2012). Nonetheless, this association is of interest from a clinical point of view as TNF inhibitors are widely used anti-inflammatory biological agents and have been successfully used to treat single AIH cases (Weiler-Normann et al. 2009).

Transforming Growth Factor (TGF)

A single report has linked polymorphism of the immunomodulatory molecule TGF-β1 to pediatric and adult AIH (Tang et al. 2012) (▶ TGF-β). However, more studies in other cohorts are required to confirm this association.

T-bet

T-bet is the key transcription factor for the Th1 cells lineage of T helper cells and is encoded by TBX21. A single report suggested a susceptibility of the TBX21 polymorphism for AIH in Chinese. Once again, further confirmation is necessary (Tang et al. 2012).

Vitamin D Receptor

Genetic associations of the receptor for 1, 25-dihydroxyvitamin D3, which is considered to act as an immunomodulator, have been found in several autoimmune diseases like multiple sclerosis, type 1 diabetes mellitus, and Graves' disease. A polymorphism at the beginning of exon 2 has been linked to AIH in European and Chinese patient cohorts (Vogel et al. 2002).

APECED

APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) is a monogenetic autosomal recessive immune disorder, which is mostly caused by mutations in the transcription factor AIRE (autoimmune regulator). A deficiency in AIRE expression results in a fundamental impairment of central tolerance induction promoted by defective deletion of autoreactive T cells in the thymus. Furthermore, the number and function of Tregs are impaired in the peripheral blood of AIRE-deficient patients (Kisand and Peterson 2011). As the name implies, APECED patients suffer from multiple autoimmune diseases mostly of the endocrine glands, and about 20 % of the patients exhibit a hepatitis resembling AIH type 2 (Lankisch et al. 2009). As indicated by AAB specificity aromatic l-amino acid decarboxylase, cytochrome P450 1A2 and ~2A6 are putative target antigens in these type of AIH (Lankisch et al. 2009; Kisand and Peterson 2011). Although the wide variability of the symptoms in APECED with AIH being an infrequent event is not understood yet, a defective central tolerance induction is thought to be a potential factor in the pathogenesis of AIH. However, APECED is a very rare disease, and mutations in AIRE are rarely found in patients with classical AIH (Vogel et al. 2001).

Molecular Mimicry/Similarity and Environmental Influences

One of the main hypotheses to explain how self-tolerance is broken to initiate AIH proposes that there is molecular mimicry or molecular similarity between pathogens and self-antigens. Pathogens would express antigens that are similar or homologue to self-structures, and the immune defense against these pathogens can progress to autoimmunity via cross-reactivity in susceptible individuals.

Consistent with this hypothesis, break of humoral tolerance is a common phenomenon in chronic viral hepatitis (e.g., hepatitis B and C), where up to 50 % of the patients have circulating AAB (ANA, SMA, or LKM). Thereby, anti-LKM-1 AAB of patients with chronic hepatitis C can even recognize the major B cell epitope of AIH type II (CYP2D6 193–212) (Longhi et al. 2010b).

Besides classical molecular mimicry, it has been suggested that due to the degeneracy of the TCR/pMHC interaction, cross-reactive T cells

can also be generated by heterologous immunity. According to the heterologous immunity model, the same TCR can recognize two peptides with unrelated amino acid sequences. Thereby, a viral infection could break tolerance against tissue antigens (Selin et al. 2004).

Danger signals leading to immune activation and tissue damage (e.g., during viral infections) have also been proposed to play a role in breaking tolerance. A vigorous immune activation, initiated by external pathogens, can overcome the immune balancing factors and allows an immune attack against self-antigens that are subsequently released during the inflammatory process. Several reports have associated viruses such as hepatitis A, B, and C virus, Epstein-Barr virus, humane herpes virus 6, and herpes simplex virus to the initiation of AIH. Although this is an attractive model, the causative contribution of infections, often subclinical and that took place months, even years before the diagnosis of AIH, is hard to prove in rare disease like AIH (Beland et al. 2009). For this reason, to date, there is no single causative infectious agent for AIH.

The genetic predisposition of the HLA DRB1 *1301 haplotype both to pediatric AIH type I and to persistence of the endemic hepatitis A virus in South America may combine the concept of molecular mimicry, autoimmune initiation via danger signals, and the ability to present autoantigens in susceptible individuals (Liberal et al. 2011).

Beside infections, some case reports have shown an association between some drugs, e.g., minocycline or some statins (atorvastatin, simvastatin), with AIH initiation supporting the altered-self hypothesis mentioned above. Once again, this hypothesis is hard to validate especially when modes of action are highly speculative and other susceptibility factors like HLA types predisposing to AIH are potential confounders in some reports (Beland et al. 2009).

Sex and Age

As many other autoimmune diseases, the female sex is overrepresented in AIH (3:1 in AIH type I; 9:1 in AIH type II). The pathophysiological mechanisms leading to this discrepancy are unknown, but several reports shed light on differences that may contribute to this phenomenon (Beland et al. 2009).

The influence of sexual hormone on AIH is suggested by several examples. AIH has the highest incidence in pediatric patients (40 % of AIH type I and 80 % of AIH type II), and a second peak is reported in postmenopausal patients. The hormonal changes during pregnancy can lead to a new diagnosis of AIH in the beginning or shortly after gravidity. Furthermore, patients in AIH remission during pregnancy often experience a postpartum disease flare (Beland et al. 2009).

Potential explanations for this clinical observation could be related to sexual differences in basic immunological processes. Women are reported to have higher T helper cell numbers and higher cytokine levels of the Th1 type and show higher immune activation after vaccination. Furthermore, estrogen can increase the production of Th1 cytokines in vitro, while testosterone can stimulate the production of the anti-inflammatory cytokine IL-10 (Beland et al. 2009).

AIH After Liver Transplantation

As AIH is often diagnosed when the patient has developed cirrhosis, liver transplantation is an important treatment option for end-stage autoimmune liver damage. Even under continued immunosuppressive therapy after liver transplantation, up to 25 % of patients suffer from a disease recurrence that displays several of the histological and biochemical hallmarks of AIH (Oo et al. 2010).

The pathogenic mechanisms of recurrence are critically discussed as alloreactivity, e.g., reactivity between mismatched HLA types from host and donor cells, and autoreactivity is hard to distinguish in clinical routine tests (Oo et al. 2010; Czaja 2012). The recurrence of AIH in transplanted patients suggests the host immune system retains reactivity against the newly transplanted liver despite continuous exposure to multiple immunosuppressants.

There are some rare reports of newly diagnosed AIH after liver transplantation subsequent to other diseases than AIH. This "de novo AIH" or "plasma cell hepatitis" affects mostly children (prevalence in children <10 %). Additionally, several studies described an overlap between de novo AIH and late cellular rejection (Oo et al. 2010). An interesting pathophysiological hypothesis in this context is the immune response against glutathione-S-transferase T1 (GSTT1), suggested by GSTT1-specific AAB, in GSTT1-deficient hosts (about 20 % of Caucasian) with a GSTT1-positive graft (about 14 % of liver transplantations). In addition to GSTT1, there might be numerous other mismatched antigens between donor and recipient that are not linked to HLA. It could be envisioned that such neoantigens could be target of an autoimmune response in genetically predisposed individuals.

Animal Models

Animal models of autoimmune liver diseases are necessary to elucidate the gaps in the understanding of the pathophysiology of AIH and have already contributed substantial inside in to initiation of autoimmunity in the liver, e.g., in terms of unspecific T cell activation by concanavalin A, immunization strategies or genetic modification of lymphocyte signaling pathways, or TCR repertoires (Hardtke-Wolenski et al. 2011). This is reviewed in detail in a separate entry of this encyclopedia.

Association with Other Syndromes

Autoimmune Liver Diseases

Primary biliary cirrhosis (PBC; ▶ Primary Biliary Cirrhosis, Overview) and primary sclerosing cholangitis (PSC; ▶ Primary Sclerosing Cholangitis: Clinical and Systemic Manifestations and Treatment) are the major autoimmune liver syndromes affecting respectively the small and the large bile ducts. Although hepatocytes are the main target of autoimmunity during AIH, the distinction between hepatic and biliary autoimmune inflammation is not as stringent as the taxonomy may imply.

Overlap of AIH and PBC Characteristics

A quarter to one-third of patients with "classical AIH," without serologic or other evidence for PBC (negativity for anti mitochondrial antibodies (AMA)), can have biliary abnormalities in liver biopsies, including destructive as well as nondestructive cholangitis or ductopenia. Reciprocally, the same percentage of PBC patients can display severe lymphocytic interface hepatitis typically observed in AIH (Boberg et al. 2011).

Both diseases display also common AAB. While ANA, SMA, and SLA are detectable in more than 30 % of PBC patients, AMA specific for the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2) that are highly specific AAB for PBC are rarely seen in AIH. In addition to anti-AMA, anti double-stranded DNA antibodies are more frequent in AIH-PBC than in PBC alone (Boberg et al. 2011).

The term "overlap syndrome" is commonly used, when patients fulfill the criteria for both disease entities.

As evidence-based criteria for the diagnosis of an overlap syndrome of AIH and PBC are missing, the percentage of AIH-PBC overlap in PBC patients varies between 5 % and 20 % depending on the diagnostic or scoring systems used. The characteristics of AIH in PBC and vice versa can develop at the same time or sequentially within months or years (Boberg et al. 2011).

Several scenarios might be proposed to explain the coexistence of interface hepatitis with PBC specific characteristics. Both portal and biliary inflammation may represent two facets of an autoimmune liver injury. An autonomous hepatocyte specific autoimmune damage in PBC beside the disease defining reactivity to PDC in the biliary epithelium seems not very likely, however. Interface hepatitis in PBC and biliary abnormalities in AIH could be caused by collateral damage. The damage of hepatocytes during PBC could release hepatocyte self-antigens, in the presence of inflammation. Similar to the danger signals caused by viral

infections, this may thus initiate a spreading of the autoimmunity to other target antigens. Why portal and not biliary inflammation responds better to immunosuppressive therapy is not completely understood (Oo et al. 2010).

Overlap of AIH and PSC Characteristics

Although portal tract inflammation associated with lymphocytic bile duct infiltration, ductular proliferation, and periductal fibrosis is a common histological characteristic of PSC, interface hepatitis can be found in up to one-third of PSC patients (Boberg et al. 2011).

The overlap of AAB profiles in AIH and PSC is substantial, as ANA, SMA, and SLA are detectable in up to 80 % of PSC patients, and perinuclear antineutrophil cytoplasmic antibodies (pANCA) can be found in 50–90 % of AIH patients (Boberg et al. 2011).

Once again, the degree of overlap depends on the scoring systems used. While PSC and PBC overlap with AIH similarly in adults (each 2–20 %), the degree of overlap in children is much higher and reaches up to 50 % of pediatric PSC patients. The majority of PSC patients (80 %) display inflammatory bowel disease (IBD) as well. Concomitant IBD in AIH is mostly diagnosed when AIH overlaps with PSC (Boberg et al. 2011).

Liver biopsies of patients displaying AIH-PSC-IBD symptoms contain a substantial infiltration (about 20 %) of T cells displaying a phenotype characteristic of gut-derived cells (α4β7+ and chemokine receptor 9+) that are rare in other liver diseases. Furthermore, in these patients, the liver endothelium expresses molecules normally displayed in the vascular bed of the small intestine (CCL25 and MADCAM-1). Thus, it is likely that other mechanisms than the ones discussed for AIH-PBC contribute to induce the AIH-PSC overlapping disease (Oo et al. 2010).

All three autoimmune liver diseases are genetically associated with the HLA locus on chromosome 6. But data regarding HLA haplotypes predisposing to overlap syndromes are inconsistent yet, because varying criteria for overlap syndromes were applied and the number of patients with overlapping syndromes is generally low (Boberg et al. 2011).

Extrahepatic Autoimmune Diseases

Several extrahepatic autoimmune diseases (EAID) are a common feature in AIH patients. In large patient cohorts, up to 40 % of AIH patients, including those that overlap with PSC and PBC, have at least one concomitant autoimmune disease mostly of the thyroid gland (Hashimoto and Graves' disease), skin (vitiligo and urticaria), joints (rheumatoid arthritis), lacrimal glands (Sjogren's syndrome), and the intestinum (e.g., celiac disease, gastritis, Crohn's disease) (Teufel et al. 2010). The cause for the disproportionate prevalence of EAID is so far unknown, but has been reported for other autoimmune diseases like type 1 diabetes. It has been shown that many patients developing autoimmune diseases share common predisposing genes. In addition to common predisposing genes, there are organ-specific genes (like insulin in type 1 diabetes). However, organ-specific predisposing genes for AIH are currently not known. There is no evidence so far that AIH patients with EAID display a more severe disease course (Teufel et al. 2010; Efe et al. 2012).

Summary

Although multiple aspects of AIH pathogenesis are scientifically accepted, the sequence of events responsible for breaking tolerance and initiating AIH is not fully understood. AIH seems to be initiated by external triggers in genetically susceptible individuals. As discussed above, immunologic susceptibility in this context is multifactorial. The ability to present relevant autoantigens is of central importance in this context as implied by the strong association with the MHC haplotypes. The association with MHC class II underlines the role of T helper in contrast to cytotoxic T cells in the immunoregulation in AIH. The role of AAB as cause or consequence of AIH remains ambiguous. In addition to these systemic factors, the exploration of the liver inflammatory local milieu will hopefully reveal further insight into the pathogenesis of AIH in the future. This might eventually lead to the development of more specific therapies for AIH patients.

References

Beland K, Lapierre P, et al. Influence of genes, sex, age and environment on the onset of autoimmune hepatitis. World J Gastroenterol. 2009;15(9):1025–34.

Boberg KM, Chapman RW, et al. Overlap syndromes: the International Autoimmune Hepatitis Group (IAIHG) position statement on a controversial issue. J Hepatol. 2011;54(2):374–85.

Czaja AJ. Autoantibodies as prognostic markers in autoimmune liver disease. Dig Dis Sci. 2010;55(8):2144–61.

Czaja AJ. Diagnosis, pathogenesis, and treatment of autoimmune hepatitis after liver transplantation. Dig Dis Sci. 2012;57(9):2248–66.

Efe C, Wahlin S, et al. Autoimmune hepatitis/primary biliary cirrhosis overlap syndrome and associated extrahepatic autoimmune diseases. Eur J Gastroenterol Hepatol. 2012;24(5):531–4.

Ferri S, Longhi MS, et al. A multifaceted imbalance of T cells with regulatory function characterizes type 1 autoimmune hepatitis. Hepatology. 2010;52(3):999–1007.

Gambineri E, Perroni L, et al.. Clinical and molecular profile of a new series of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: inconsistent correlation between forkhead box protein 3 expression and disease severity. J Allergy Clin Immunol 2008; 122(6): 1105–1112 e1.

Greeley SA, Katsumata M, et al. Elimination of maternally transmitted autoantibodies prevents diabetes in nonobese diabetic mice. Nat Med. 2002;8(4):399–402.

Hardtke-Wolenski M, Noyan F, et al. Requirements and challenges of a preclinical autoimmune hepatitis mouse model. Dig Dis. 2011;29(4):402–10.

Hardtke-Wolenski M, Fischer K, et al. Genetic predisposition and environmental danger signals initiate chronic autoimmune hepatitis driven by CD4+ T cells. Hepatology 2013; doi: 10.1002/hep.26380.

Kirk AP, Jain S, et al. Late results of the Royal Free Hospital prospective controlled trial of prednisolone therapy in hepatitis B surface antigen negative chronic active hepatitis. Gut. 1980;21(1):78–83.

Kisand K, Peterson P. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy: known and novel aspects of the syndrome. Ann N Y Acad Sci. 2011;1246:77–91.

Lankisch TO, Jaeckel E, et al. The autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy or autoimmune polyglandular syndrome type 1. Semin Liver Dis. 2009;29(3):307–14.

Liberal R, Longhi MS, et al. Pathogenesis of autoimmune hepatitis. Best Pract Res Clin Gastroenterol. 2011;25(6):653–64.

Liberal R, Grant CR, et al. The impaired immune regulation of autoimmune hepatitis is linked to a defective Galectin-9/Tim-3 pathway. Hepatology. 2012;56(2):677–86.

Lleo A, Shimoda S, et al. Primary biliary cirrhosis and autoimmune hepatitis: apotopes and epitopes. J Gastroenterol. 2011;46(Suppl 1):29–38.

Longhi MS, Ma Y, et al. Aetiopathogenesis of autoimmune hepatitis. J Autoimmun. 2010a;34(1):7–14.

Longhi MS, Ma Y, et al. Adaptive immunity in autoimmune hepatitis. Dig Dis. 2010b;28(1):63–9.

Manns MP, Woynarowski M, et al. Budesonide induces remission more effectively than prednisone in a controlled trial of patients with autoimmune hepatitis. Gastroenterology. 2010;139(4):1198–206.

Mix H, Weiler-Normann C, et al. Identification of CD4 T-cell epitopes in soluble liver antigen/liver pancreas autoantigen in autoimmune hepatitis. Gastroenterology. 2008;135(6):2107–18.

Oliveira LC, Porta G, et al. Autoimmune hepatitis, HLA and extended haplotypes. Autoimmun Rev. 2011;10(4):189–93.

Oo YH, Hubscher SG, et al. Autoimmune hepatitis: new paradigms in the pathogenesis, diagnosis, and management. Hepatol Int. 2010;4(2):475–93.

Peiseler M, Sebode M, et al. FOXP3+ regulatory T cells in autoimmune hepatitis are fully functional and not reduced in frequency. J Hepatol. 2012;57(4): 933–934.

Sakaguchi S, Miyara M, et al. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol. 2010;10(7):490–500.

Selin LK, Cornberg M, et al. CD8 memory T cells: cross-reactivity and heterologous immunity. Semin Immunol. 2004;16(5):335–47.

Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. Immunity. 2009;30(5):636–45.

Strassburg CP, Manns MP. Autoantibodies and autoantigens in autoimmune hepatitis. Semin Liver Dis. 2002;22(4):339–52.

Tang J, Zhou C, et al. Association of polymorphisms in non-classic MHC genes with susceptibility to autoimmune hepatitis. Hepatobiliary Pancreat Dis Int. 2012;11(2):125–31.

Teufel A, Weinmann A, et al. Concurrent autoimmune diseases in patients with autoimmune hepatitis. J Clin Gastroenterol. 2010;44(3):208–13.

Vogel A, Liermann H, et al. Autoimmune regulator AIRE: evidence for genetic differences between autoimmune hepatitis and hepatitis as part of the autoimmune polyglandular syndrome type 1. Hepatology. 2001;33(5):1047–52.

Vogel A, Strassburg CP, et al. Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. Hepatology. 2002;35(1):126–31.

Wachter B, Kyriatsoulis A, et al. Characterization of liver cytokeratin as a major target antigen of anti-SLA antibodies. Hepatology. 1990;11(2):232–9.

Weiler-Normann C, Wiegard C, et al. A case of difficult-to-treat autoimmune hepatitis successfully managed by TNF-alpha blockade. Am J Gastroenterol. 2009;104(11):2877–8.

Autoimmune Kidney Disease and Pregnancy

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Synonyms

Lupus nephritis; Maternal and fetal morbidity and mortality; Pregnancy; Pregnancy complications; Systemic lupus erythematosus

Definition

A number of autoimmune kidney diseases, including systemic lupus erythematosus (SLE), systemic sclerosis, and atypical hemolytic-uremic syndrome, may be present during pregnancy. Pregnancy in patients with these conditions must be considered from three different perspectives: the effect of pregnancy on disease activity/progression, effects of disease/treatments on the fetus, and health of the mother during pregnancy and after delivery.

Introduction

The optimal management of patients with autoimmune kidney disease who become pregnant is crucially dependent on a multispecialty team consisting of a nephrologist, rheumatologist, and obstetrician. Adverse fetal outcomes (spontaneous abortion, prematurity, intrauterine growth restriction) as well as maternal outcomes (preeclampsia, renal disease flare) are common, particularly in patients with active renal disease at conception. Consequently, irrespective of the underlying etiology, remission of renal disease for $>$ 6 months prior to conception is recommended. The major goal of immunosuppressive therapy in pregnancy is control of disease activity with medications that are relatively safe for a growing fetus. While worsening proteinuria in the third trimester may occur in all proteinuric renal diseases, the differential diagnosis includes a renal disease flare and preeclampsia, a pregnancy-specific condition clinically characterized by hypertension and proteinuria. Due to the elevated risk for renal disease flares postpartum, the affected women need to be followed after delivery.

Systemic Lupus Erythematosus and Lupus Nephritis

The most common, hence the most studied, autoimmune disease in pregnancy is systemic lupus erythematosus (SLE), a multisystem autoimmune connective tissue disorder that predominantly affects women of childbearing age. SLE does not directly affect fertility; as such, pregnancy is a frequent occurrence. Although initial studies suggested an association between SLE and poor pregnancy outcomes, improved outcomes have been observed more recently, including live birth rates of up to 85 %. Similarly, the mortality associated with SLE has declined (Smyth and Garovic 2009). Consequently, a growing number of women with SLE are seeking to become pregnant.

Fertility

Data suggest that fertility is preserved in patients with SLE in the absence of advanced renal insufficiency, defined as a serum creatinine $\geq$3 mg/dL, or previous therapy with cytotoxic alkylating agents, including cyclophosphamide. As not all pregnancies in patients with SLE are planned, it is proposed that lupus does not affect fertility and that fertility rates are comparable to the general population (Smyth and Garovic 2009). In patients with advanced renal

insufficiency, renal transplantation may restore fertility; no significant differences in pregnancy outcomes have been reported when compared to non-SLE transplant recipients.

Pregnancy Outcomes

Pregnancy outcomes in women with SLE have improved significantly since the original reports. There has been a decrease in the rate of fetal loss from 40 %, in 1960–1965, to 17 %, in 2000–2003 (Smyth and Garovic 2009). Although outcomes have improved, reported rates of maternal and fetal morbidity vary widely. Postulated causes include variations in ethnicity, socioeconomic status, measures of SLE activity/diagnosis, and the heterogeneity of study designs (Petri 1997). A US study, including 13,555 pregnancies in patients with lupus nephritis (LN), showed a 20-fold increase in maternal mortality in those with LN compared to the general population, with odds ratios of 1.7 for caesarean section, 2.4 for preterm labor, and 3.0 for preeclampsia. Meta-analyses of pregnancy outcomes in patients with SLE and LN reported maternal (e.g., lupus flare in 25.6 %, preeclampsia in 7.6 %) and fetal complications (e.g., spontaneous abortion in 16.0 % and intrauterine growth retardation [IUGR] in 12.7 %). Regression analysis showed that active nephritis was associated with premature birth and hypertension. In addition, a previous history of nephritis was associated with hypertension. The presence of antiphospholipid antibodies was also associated with hypertension, premature birth, and induced abortion (Smyth et al. 2010).

Fetal Complications

Although the majority of patients with SLE sustain pregnancy with minimal complications, compared to the general population, the overall risk for fetal loss is increased (Smyth and Garovic 2009). Rates of 11.5–46 % are reported in those with inactive renal disease, but >50 % in the presence of active renal disease (Wagner et al. 2009) (Fig. 1). This risk is even higher if previous pregnancies were affected by complications, in particular, previous pregnancy loss (Smyth and Garovic 2009).

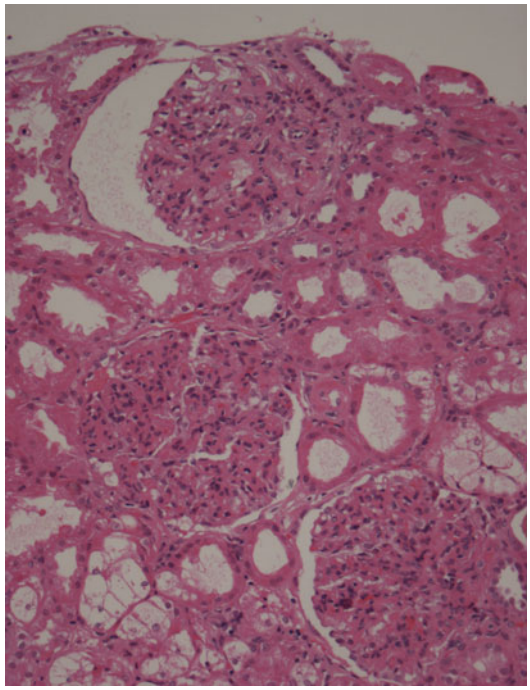
Spontaneous abortion, defined as the spontaneous loss of a fetus before 20 weeks of gestation, occurs more often in patients with SLE. When induced abortions are excluded, the rate of spontaneous abortion may be as high as 16.0 % (Smyth et al. 2010).

Preterm birth, defined as a live birth occurring before 37 weeks of gestation, occurs more frequently in patients with SLE. Rates of 34–50 % have been reported, consistent with a rate of 39.4 % from meta-analyses. Intrauterine growth restriction (IUGR), defined as an estimated birth weight less than the 10th percentile for gestational age, occurs in 12.7 % of SLE pregnancies (Smyth et al. 2010). Hypertension, particularly during early pregnancy, correlates strongly with lower birth weight. It is thought that the development of vascular necrotic lesions in the placentas of women with SLE contributes to the pathogenesis of spontaneous abortion and IUGR (Smyth and Garovic 2009).

Neonatal lupus is a passively acquired autoimmune disorder occurring in babies born to mothers with autoimmune conditions, including SLE, due to passage of maternal anti-Ro/SSA into the fetal circulation. It is associated with fetal congenital heart block, but has not been associated with any significant effects on other pregnancy outcomes. Congenital heart block carries a high mortality rate of 19 %, and up to 64 % of surviving infants will require a pacemaker. The incidence of neonatal lupus in the offspring of mothers who are anti-Ro/SSA positive is reported to be 1.5–2.0 % (Smyth and Garovic 2009).

Maternal Complications

Rates of maternal hypertension in pregnant lupus patients vary widely, with rates as high as 43 % reported; meta-analyses reported a rate of 16.3 % (Smyth et al. 2010). Hypertension in pregnancy is defined as a systolic blood pressure greater than 140 mmHg and/or a diastolic blood pressure greater than 90 mmHg, and is referred to as gestational hypertension when documented after 20 weeks of gestation. The diagnosis of preeclampsia is entertained when the onset of



Autoimmune Kidney Disease and Pregnancy, Fig. 1 A 19-year-old, gravida two, para one, presented with cough and hemoptysis at 8 weeks of gestation. Past medical history was significant for SLE diagnosed at age 12, and preeclampsia with her previous pregnancy, leading to labor induction at 8 months of gestation. Five months prior to pregnancy, due to worsening renal function and proteinuria, renal biopsy showed diffuse proliferative LN. Mycophenolate mofetil and prednisone were commenced, but when pregnancy was confirmed, mycophenolate mofetil was discontinued. The patient subsequently presented with shortness of breath, fever, hypoxia, and hemoptysis. Antinuclear antibody (ANA) was elevated to 9.1 U, creatinine increased from <1.0 to 1.6 mg/dL, and urine studies showed an active urine sediment, with dysmorphic red blood cells and proteinuria of 6 g in 24 h. As chest x-ray showed diffuse pulmonary infiltrates, bronchoscopy was performed and showed diffuse pulmonary hemorrhage. Treatment with prednisone, azathioprine, and plasma exchange was initiated. Unfortunately, the patient developed vaginal bleeding and subsequent pregnancy loss. Mycophenolate mofetil was restarted, with improvement in renal function and a follow-up creatinine of 0.8 mg/dL.
This case demonstrates poor pregnancy outcomes in patients with active LN. The necessity to modify and/or discontinue immunosuppressive agents (in this case, mycophenolate mofetil) may contribute to exacerbation of disease activity and, consequently, poor outcomes

hypertension after 20 weeks gestation is accompanied by de novo proteinuria > 300 mg/day.

Preeclampsia may affect up to 7.6 % of lupus pregnancies (Smyth et al. 2010) and may be difficult to distinguish from a lupus flare (Table 1). The diagnosis of preeclampsia is characterized by negative or unchanged lupus serologies, absence of extrarenal manifestations of SLE, proteinuria (in the absence of an active urine sediment), and elevated serum uric acid levels. Decreased urinary output, worsening renal function, low serum complement levels, and increased titers of anti-DNA antibodies are more characteristic of a lupus flare. Complement levels may not be helpful in differentiating a lupus flare from preeclampsia; instead, the CH50/Ba ratio may be more useful. In active lupus, CH50 levels remain normal, but elevations of complement split products, which include Ba, result in a high ratio of CH50/Ba (Smyth and Garovic 2009). Newer biomarkers, including antiangiogenic markers, such as soluble vascular endothelial receptor 1, sFlt-1 (Maynard et al. 2003), may help with this differentiation in the future. Preeclampsia is more common in patients with antiphospholipid antibodies, preexisting diabetes mellitus, or with previous histories of preeclampsia. Eclampsia, defined as generalized convulsions and/or coma occurring in the setting of preeclampsia, in the absence of other neurological conditions, is a rare but significant complication and affects <1 % of lupus pregnancies (Smyth et al. 2010).

Maternal mortality in SLE patients is a relatively rare complication in the developed world. Its true rate is difficult to assess. There may be a reporting bias favoring the publication of cases of maternal death, and the true number of lupus pregnancies (the denominator) is unknown. Meta-analyses have reported a rate of <1 % (Smyth et al. 2010). Importantly, a recent review of the literature identified seventeen cases of maternal mortality in pregnancies with LN. In all cases, death occurred in the setting of active renal disease, with infection and SLE complications being the two most common

causes, accounting for 41.2 % and 29.4 % of all deaths, respectively (Ritchie et al. 2012). This emphasizes the importance of planning pregnancy in patients with SLE, judicious use of immunosuppressive therapy, and the need for expert monitoring.

SLE and Renal Flares

During pregnancy, the maternal immune system adapts to facilitate the growth of the semi-allogeneic fetus. This includes the inhibition of cytokine production by type-1 helper cells (Th1) of the cellular immune system and enhanced cytokine production by type-2 helper cells (Th2) from the humoral immune system (Tower et al. 2011). This upregulation of Th2 cytokines may increase the risk of diseases mediated by Th2 cells, including SLE. In addition, delivery/termination of pregnancy may result in further changes in this immune state, toward a more stimulated state, which may also increase the risk of flare (Smyth and Garovic 2009).

Studies of the effects of pregnancy on SLE/LN disease activity have provided conflicting data when comparing flare rates between pregnant and nonpregnant patients (Smyth et al. 2010). In contrast, studies that have used a patient's own nonpregnant course for comparison have commonly demonstrated an increase in disease activity during pregnancy (Smyth and Garovic 2009). Another factor that may have contributed to the differing results with respect to the risk of SLE flare in pregnancy relates to the varying definitions of flare being used in published studies. Some features used to diagnose flare in the nonpregnant state may not be valid during pregnancy, as these may represent either normal physiologic changes or pregnancy-related complications other than lupus flare. One notable example is the worsening of proteinuria that commonly occurs toward the end of pregnancy in women with preexisting proteinuric renal diseases, which, in the case of LN, may be confused with LN flare and/or preeclampsia. To overcome this problem of misclassification, three scoring systems, which are used to measure ongoing SLE activity and flare, have been modified to take into account the physiological and clinical changes of pregnancy that can confound the evaluation of lupus activity (Ruiz-Irastorza et al. 2004).

In summary, significant differences in study designs and definitions of flare might have contributed to the conflicting data regarding the risk of SLE flare in pregnancy. As the risk for flare may be as high as 70 %, close monitoring of these patients, with monthly assessment of disease activity, is recommended (Petri 1998).

Lupus Nephritis

Up to 75 % of patients with SLE have clinically evident renal disease, which is one of the American College of Rheumatology criteria used for the classification of SLE. Active renal involvement is defined as the presence of an active urine sediment (greater than five red and white blood cells per high-power field and/or one or more cellular casts) and/or proteinuria > 0.5 g/day, with or without an elevation in serum creatinine. Pregnancies in patients with stable renal involvement generally have good outcomes, particularly if pregnancy is planned and conception occurs after a period of 12–18 months (Urowitz et al. 1993) (minimum of 6 months) of disease remission. In contrast, those with active disease at conception are associated with severe complications and deterioration in renal function (Hayslett and Lynn 1980).

The relationship between lupus nephritis and pregnancy outcome is complex. Pregnancy may have adverse short- and long-term effects on kidney function, including an increased risk for LN flare and progression to end-stage renal disease. Similar to other renal diseases in pregnancy, the risk for progression is determined in part by the severity of the underlying renal disease and is increased for patients with creatinine values greater than 1.4 mg/dL (Hou 1999). Underlying renal disease, in turn, places these pregnancies at higher risk for

maternal and fetal complications, including spontaneous abortion, premature delivery, intrauterine growth retardation, and superimposed preeclampsia.

Management of SLE and Pregnancy

Lupus pregnancies are high risk, and care should be provided by obstetricians and internal medicine specialists with an interest in SLE. Cornerstones of management include preconception counseling, close monitoring, and the provision of expert care throughout. Prior to pregnancy, it is advised that lupus be quiescent to achieve the best pregnancy outcomes. Accordingly, some patients will require maintenance immunosuppression during pregnancy. As many of these agents can cross the placenta and potentially cause fetal harm, the immunosuppressive regimen needs to be carefully reviewed and modified prior to pregnancy.

Once pregnancy is confirmed, the initial consultation should include a thorough physical examination, blood pressure monitoring, urinalysis, and 24-h urine protein collection. Investigations should include complete blood count (to rule out anemia, leukopenia, or thrombocytopenia) and serologies for autoantibodies, including anti-Ro/SSA and anti-La/SSB (due to risk of neonatal lupus), and antiphospholipid antibodies. Markers of disease activity should be reassessed monthly during the first two trimesters, with more frequent monitoring in the third trimester. Excluding antiphospholipid antibodies, those with increased serological activity in the absence of symptoms do not require the immediate initiation of treatment; instead, they are considered higher risk and should be followed up more closely.

If a renal flare occurs during pregnancy, it is important to differentiate LN from preeclampsia and its severe form, HELLP syndrome (Weinstein 1982), which is characterized by **h**emolysis, **e**levated **l**iver enzymes, and **l**ow **p**latelet count (Table 1). Superimposed preeclampsia may be particularly difficult to diagnose in a hypertensive patient with proteinuria predating pregnancy. This distinction is critical as a LN flare is managed with immunosuppression, whereas delivery, even remote from term, is indicated for severe and superimposed preeclampsia. In select cases, renal biopsy may be safely performed in patients with good blood pressure control and normal coagulation parameters (Smyth and Garovic 2009) to make a definitive diagnosis and to inform treatment decisions (Table 2). Typically, renal biopsy is not advisable after 32 weeks of gestation.

Autoimmune Kidney Disease and Pregnancy, Table 1 Differentiation of preeclampsia, HELLP syndrome, and lupus nephritis flare

	Preeclampsia	HELLP syndrome	LN flare
Timing in pregnancy	After 20 weeks	After 20 weeks	Any time during pregnancy
Complement (C3, C4)	Normal	Normal	Typically decreased
Thrombocytopenia	Absent	Present	Present
Neutropenia	Absent	Absent	Present
Active urine sediment (red blood cell casts)	Absent	Absent	Present
Other organ involvement	Absent	Absent	Present
Anti-double-stranded DNA antibodies Direct Coombs antiplatelet antibodies	Absent	Absent	Present
Anti C1q antibodies	Normal	Normal	May be high
Abnormal liver function tests	Absent	Present	Absent
Serum uric acid	Increased	Increased	Normal

Antiphospholipid Antibodies/Syndrome

Antiphospholipid antibodies, including lupus anticoagulant and anticardiolipin antibodies, are autoantibodies that bind to cardiolipin and/or β2 glycoprotein I bound to phospholipids. The presence of these antibodies, in association with clinical features such as venous or arterial thromboses, recurrent miscarriages, livedo reticularis, cutaneous ulcers, cardiac valvular disease, and thrombocytopenia, constitutes

Autoimmune Kidney Disease and Pregnancy, Table 2 Anti-inflammatory and immunosuppressive drugs in pregnancy

Drug name	Comments	FDA class[a]	Breast-feeding[b]
Corticosteroids	Risks of use often outweighed by risk of underlying disease Potential risks for orofacial clefts and premature birth	C	Usually compatible with breast-feeding
Hydroxychloroquine	Considered safe in pregnancy at 200–400 mg/day. Discontinuation during pregnancy associated with increased risk of lupus flare. May use for maintenance or mild flares	Not assigned	Usually compatible with breast-feeding
Nonsteroidal anti-inflammatory drugs (NSAID)	Avoidance after 30 weeks of gestation because of the effects of NSAID-related prostaglandin inhibition on the fetal cardiovascular system (closure of ductus arteriosus)	C	Usually compatible with breast-feeding
Cyclosporine	Can be maintained in pregnancy at lowest effective dose No significant increase in rate of congenital malformations	C	Not recommended
Tacrolimus	Can be maintained in pregnancy at lowest effective dose Potential risk of neonatal hyperkalemia and renal dysfunction	C	Not recommended
Rituximab	Limited safety data; may alter fetal and neonatal B-cell development	C	Not recommended
Azathioprine	May use for flare during pregnancy. Consider as alternative to mycophenolate. Avoid doses >1.5–2 mg/kg/day due to risk of suppressed neonatal hematopoiesis	D	Not recommended
Mycophenolate mofetil	Contraindicated during pregnancy due to teratogenicity (ocular, facial, and digits)	D	Not recommended
Cyclophosphamide	Useful when maternal disease is life-threatening High risk of fetal loss but less pronounced in more recent studies	D	Not recommended
Methotrexate	High risk of miscarriage and congenital abnormality Treatment should be withdrawn 3 months before pregnancy	X	Not recommended

[a]According to the Food and Drug Administration pregnancy ratings
[b]According to either the World Health Organization and/or Thomson lactation ratings

antiphospholipid syndrome (APS) (Rosove and Brewer 1992). Antiphospholipid antibodies can be seen in patients with SLE and other connective tissue diseases (CTD) (secondary APS), but may also occur in patients without other identifiable disease (primary APS).

Both the presence of antiphospholipid antibodies and antiphospholipid syndrome are frequently seen in association with SLE and are reported to occur in almost one in four of these pregnancies (Smyth et al. 2010). The main complication associated with these antibodies is fetal loss, which generally occurs after 10 weeks gestation (Smyth and Garovic 2009). As the risk of pregnancy loss has been correlated with the number of positive tests for different antiphospholipid antibodies (Ruffatti et al. 2009), screening for the presence of these antibodies during the initial evaluation of all lupus pregnancies is recommended. Several studies have demonstrated an increased relative risk of preeclampsia in patients with antiphospholipid antibodies or APS (do Prado et al. 2010).

The prevalence of renal involvement in patients with APS is unknown (D'Cruz 2009). Patients with antiphospholipid syndrome may develop thromboses in small diameter renal vessels leading to areas of cortical ischemia and/or necrosis and renal infarction. These patients may present with hypertension, variable degrees of proteinuria, and renal insufficiency. Renal biopsy typically demonstrates thrombotic microangiopathy. Patients with antiphospholipid antibodies are also at risk for the development of renal vein thrombosis, which frequently presents with sudden onset of severe, often nephrotic range, proteinuria. The thrombosis of the renal vein can often be confirmed by ultrasound studies. The risk of these

complications may be increased due to the hypercoagulable state of pregnancy.

The mainstay of management of antiphospholipid antibody/syndrome is anticoagulation, which appears to improve both maternal and fetal outcomes in patients with APS. In the nonpregnant state, patients are usually started on heparin and then transitioned to warfarin, with a target INR of 2.5–3.5. The use of warfarin is contraindicated during pregnancy, as it crosses the placenta resulting in teratogenicity and life-threatening hemorrhage in the infant during the perinatal period. Instead, most centers use aspirin and/or low-molecular-weight heparin. Immunosuppression is reserved for those who, in addition to APS, have active SLE, as glucocorticoids have been associated with increased prematurity without a reduction in pregnancy loss rate (Smyth and Garovic 2009).

Women with a history of antiphospholipid syndrome and arterial thrombotic events should be advised against pregnancy due to high risks, not only for pregnancy losses, but also for stroke and related maternal morbidity and mortality (Petri 1997). For these women, surrogacy can be considered as a means of achieving parenting goals without taking risks related to their own child bearing.

Systemic Sclerosis/Scleroderma

Scleroderma is up to five times more prevalent in women than in men, with a mean age of onset at age 40, and thus not commonly seen in women of childbearing age (Steen 1999). Up to 10 % of all scleroderma patients develop scleroderma renal crisis, a condition that presents with an abrupt onset of renin-mediated hypertension and renal failure in patients with diffuse sclerotic skin changes. It remains unclear as to whether the risk for scleroderma renal crisis is increased in pregnancy. Adequate treatment of this condition involves angiotensin-converting enzyme (ACE) inhibitors and thus poses a major challenge in pregnant patients, as these medications, along with angiotensin II receptor blockers (ARB), are contraindicated during pregnancy. However, their use in pregnancy may be justified as a life-saving treatment for severe hypertension in association with scleroderma renal crisis. As renal crisis is most common within 5 years of disease onset, women with scleroderma should be advised that delaying pregnancy may lessen the risk of renal crisis. Patients with the limited form of scleroderma, known as CREST (calcinosis, Raynaud's, esophageal dysmotility, sclerodactyly, telangiectasia) syndrome, usually do not have renal involvement and may do well in pregnancy, save for Mallory-Weiss tears that may occur in patients with esophageal disease who vomit during pregnancy.

Atypical Hemolytic-Uremic Syndrome (HUS)

Atypical hemolytic-uremic syndrome (HUS) is characterized by hemolytic anemia, thrombocytopenia, and renal impairment that occur as a consequence of uncontrolled activation of the complement system. In contrast, classical HUS occurs secondary to infection with bacteria that produce Shiga-like toxin (Noris and Remuzzi 2009). Complement abnormalities occur due to inactivating mutations of the factors that downregulate complement activation (factor H, factor I, or membrane cofactor protein), activating gain-of-function mutations in factor B or C3 leading to complement activation, and as a result of acquired anti-factor H antibodies. The pregnancy state may trigger abnormal complement activation leading to atypical HUS. In a recent series of 100 patients with atypical HUS, 21 women developed atypical HUS with pregnancy, with 79 % occurring postpartum (Fakhouri et al. 2010), with complement abnormalities present in 18 of the 21 patients. These patients were at an increased risk for fetal loss and preeclampsia, and 76 % reached end-stage renal disease by last follow-up. Recent work suggests that mutations in the genes coding for complement regulatory proteins may increase the risk for preeclampsia in other disease entities, such as SLE and/or APL antibodies (Salmon et al. 2011).

Membranous Nephropathy

Membranous nephropathy (MN) is one of the most common causes of nephrotic syndrome and is characterized by diffuse thickening of the glomerular basement membrane and subepithelial electron-dense deposits. The majority of cases are idiopathic, and the M-type phospholipase A2 receptor (PLA2R), expressed on glomerular podocytes, has recently been identified as a major antigen in human idiopathic MN (Beck et al. 2009). In young women of childbearing age, it is most commonly associated with SLE, with other etiologies including drug exposure (such as penicillamine, NSAIDs, and biologic agents), hepatitis B, hepatitis C, malignancy, and syphilis. In the nonpregnant state, treatments include ACE inhibitors, lipid-lowering therapy, anticoagulation, immunosuppression (including cyclophosphamide, glucocorticoids, or calcineurin inhibitors), and rituximab. ACE inhibitors during the first semester of pregnancy are associated with increased risks of cardiovascular or neurological malformations (Cooper et al. 2006), and during the second and third trimesters, may impair fetal renal function or result in poor pulmonary maturation and the development of limb contractures and skeletal malformations (Pryde et al. 1993). As such, ACE inhibitors are contraindicated during pregnancy.

Vasculitides

Takayasu's arteritis commonly affects women with age of onset of between 10 and 40 years. Subsequently, it is the second most common form of vasculitis in pregnancy, after lupus. It typically presents as a multisystem disease with renal involvement, hypertension, stroke, and heart failure. The risk of preeclampsia is increased in patients with abdominal aortic and renal artery involvement. In contrast to lupus and Takayasu's, other forms of vasculitides are more common in men than in women and typically present after the reproductive period. Subsequently, there are very few published studies, and those that are available present limited information. Briefly, ANCA vasculitides, including granulomatosis with polyangiitis (previously known as Wegener's granulomatosis) and microscopic polyangiitis, may present for the first time either during pregnancy or postpartum. In a few cases, cyclophosphamide has been used to treat flares during pregnancy with the subsequent successful deliveries of healthy babies. For the most part, patients with inactive disease at conception fare well, leading to the current recommendation that a stable remission of 6 months duration be obtained prior to conception. In addition, as steroid withdrawal can predispose to Churg-Strauss syndrome, high-dose steroids for fetal lung maturation should be used judiciously (Gordon 2004).

Conclusion

A number of autoimmune conditions are prevalent in women of childbearing age, and the occurrence of pregnancy in such patients is not infrequent. Both maternal and fetal complications may occur if the disease is active at conception. Although individual disease management is subspecialized, it is recommended that pregnancy in such patients be planned to occur when disease has been in remission for a minimum of 6 months prior to conception. Immunosuppressive agents should be optimized prior to conception, and should a flare occur during pregnancy, agents including corticosteroids, hydroxychloroquine, cyclosporine, tacrolimus, and azathioprine are safe for use. NSAIDs are best avoided after 30 weeks of gestation; rituximab and cyclophosphamide use is discouraged; methotrexate and mycophenolate mofetil are not safe for use.

Cross-References

- ▶ Antiphospholipid Syndrome Treatment
- ▶ Antiphospholipid Syndrome, Clinical Manifestations
- ▶ Lupus Nephritis, Diagnosis and Treatment

- ▶ Pregnancy in Systemic Lupus Erythematosus
- ▶ Scleroderma-Like Conditions of the Skin
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Systemic Lupus Erythematosus, Congenital Heart Block and Neonatal Lupus
- ▶ Systemic Lupus Erythematosus, Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Vasculitis and the Kidney

References

Beck Jr LH, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361(1):11–21.

Cooper WO, Hernandez-Diaz S, Arbogast PG, Dudley JA, Dyer S, Gideon PS, Hall K, Ray WA. Major congenital malformations after first-trimester exposure to ACE inhibitors. N Engl J Med. 2006;354(23):2443–51.

D'Cruz D. Renal manifestations of the antiphospholipid syndrome. Curr Rheumatol Rep. 2009;11(1):52–60.

do Prado AD, Piovesan DM, Staub HL, Horta BL. Association of anticardiolipin antibodies with preeclampsia: a systematic review and meta-analysis. Obstetrics and Gynecology. 2010;116(6):1433–43.

Fakhouri F, Roumenina L, Provot F, Sallee M, Caillard S, Couzi L, Essig M, Ribes D, Dragon-Durey MA, Bridoux F, Rondeau E, Fremeaux-Bacchi V. Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. J Am Soc Nephrol. 2010;21(5):859–67.

Gordon C. Pregnancy and autoimmune diseases. Best Pract Res Clin Rheumatol. 2004;18(3):359–79.

Hayslett JP, Lynn RI. Effect of pregnancy in patients with lupus nephropathy. Kidney Int. 1980;18(2):207–20.

Hou S. Pregnancy in chronic renal insufficiency and end-stage renal disease. Am J Kidney Diseases. 1999;33(2):235–52. The official journal of the National Kidney Foundation.

Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Selke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111(5):649–58.

Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(17):1676–87.

Petri M. Hopkins Lupus Pregnancy Center: 1987 to 1996. Rheum Dis Clin North Am. 1997;23(1):1–13.

Petri M. Pregnancy in SLE. Baillieres Clin Rheumatol. 1998;12(3):449–76.

Pryde PG, Sedman AB, Nugent CE, Barr Jr M. Angiotensin-converting enzyme inhibitor fetopathy. J Am Soc Nephrol. 1993;3(9):1575–82.

Ritchie J, Smyth A, Tower C, Helbert M, Venning M, Garovic VD. Maternal deaths in women with Lupus Nephritis: a review of published evidence. Lupus. 2012;21(5):534–41.

Rosove MH, Brewer PM. Antiphospholipid thrombosis: clinical course after the first thrombotic event in 70 patients. Ann Intern Med. 1992;117(4):303–8.

Ruffatti A, Tonello M, Cavazzana A, Bagatella P, Pengo V. Laboratory classification categories and pregnancy outcome in patients with primary antiphospholipid syndrome prescribed antithrombotic therapy. Thromb Res. 2009;123(3):482–7.

Ruiz-Irastorza G, Khamashta MA, Gordon C, Lockshin MD, Johns KR, Sammaritano L, Hughes GR. Measuring systemic lupus erythematosus activity during pregnancy: validation of the lupus activity index in pregnancy scale. Arthritis Rheum. 2004;51(1):78–82.

Salmon JE, Heuser C, Triebwasser M, Liszewski MK, Kavanagh D, Roumenina L, Branch DW, Goodship T, Fremeaux-Bacchi V, Atkinson JP. Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. PLoS Med. 2011;8(3):e1001013.

Smyth A, Garovic VD. Systemic lupus erythematosus and pregnancy. Minerva Urol Nefrol. 2009;61(4):457–74.

Smyth A, Oliveira GH, Lahr BD, Bailey KR, Norby SM, Garovic VD. A systematic review and meta-analysis of pregnancy outcomes in patients with systemic lupus erythematosus and lupus nephritis. Clin J Am Soc Nephrol. 2010;5(11):2060–8.

Steen VD. Pregnancy in women with systemic sclerosis. Obstet Gynecol. 1999;94(1):15–20.

Tower C, Crocker I, Chirico D, Baker P, Bruce I. SLE and pregnancy: the potential role for regulatory T cells. Nat Rev Rheumatol. 2011;7(2):124–8.

Urowitz MB, Gladman DD, Farewell VT, Stewart J, McDonald J. Lupus and pregnancy studies. Arthritis Rheum. 1993;36(10):1392–7.

Wagner SJ, Craici I, Reed D, Norby S, Bailey K, Wiste HJ, Wood CM, Moder KG, Liang KP, Liang KV, Rose C, Rozkos T, Sitina M, Grande JP, Garovic VD. Maternal and foetal outcomes in pregnant patients with active lupus nephritis. Lupus. 2009;18(4):342–7.

Weinstein L. Syndrome of hemolysis, elevated liver enzymes, and low platelet count: a severe consequence of hypertension in pregnancy. Am J Obstet Gynecol. 1982;142(2):159–67.

Autoimmune Myocarditis and Pericarditis

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Synonyms

CAR, coxsackievirus and adenovirus receptor; CD, cluster of differentiation; cMRI, cardiac magnetic resonance imaging; DCM, dilated cardiomyopathy; DNA, deoxyribonucleic acid; ECG, electrocardiography; EKG, echocardiography; EMB, endomyocardial biopsy; GCM, giant cell myocarditis; HLA, human leukocyte antigen; IFN, interferon; IgG, immunoglobulin G; IgM, immunoglobulin M; IL, interleukin; LV, left ventricle; PCR, polymerase chain reaction; RNA, ribonucleic acid; RV, right ventricle; Th1/Th2, T helper cell 1/2; TNF-α, tumor necrosis factor alpha; TnI, cardiac troponin I

Definition

Myocarditis: Myocarditis is an inflammatory disease of the heart muscle (myocardium) that can result from a variety of causes.

Pericarditis: Pericarditis is an inflammation of the two layers of the thin, saclike membrane that surrounds the heart and major blood vessels. This membrane is called the pericardium, so the term pericarditis means inflammation of the pericardium.

Myocarditis

Myocarditis is defined by the inflammation of the heart muscle composed of an inflammatory infiltrate and myocyte necrosis resulting in damage of the heart muscle with decrease in myocardial function. Because of inflammation and muscle damage, the heart is unable to respond to the increase in volume and impaired systolic function of one or both ventricles through an increase in contractility (Starling's law).

Myocarditis can be classified by etiology, histology, immunohistology, clinicopathological, and clinical criteria (Table 1). The diagnosis of myocarditis is still difficult given the vast scale of its clinical presentations ranging from almost asymptomatic to cardiac dysrhythmias and heart failure with the need of heart transplantation. As most cases are characterized by a subclinical course, many patients do not ever see a doctor while acutely suffering from myocarditis. In more than half of the patients, there is spontaneous healing and no symptoms remain. But, the destruction of the myocardium in conjunction with tissue remodelling can also lead to ventricular dysfunction and dilated cardiomyopathy in some patients, even when asymptomatic. Acute myocarditis has been shown to be the cause of sudden death in up to 12 % of cases in individuals less than 40 years old.

The Dallas criteria (presence of inflammatory cellular infiltrate with or without associated myocyte necrosis and damage not characteristic of ischemic tissue) were proposed in 1986 (Aretz et al. 1987; Cooper Jr 2009) to provide a histopathological classification for the diagnosis of myocarditis. Because of their limitation (low sensitivity that is part due to sampling error, variability in expert interpretation, lack of prognostic value, variance with treatment outcomes), newer histological criteria using cell-specific immunoperoxidase stains for surface antigens like CD-3, CD-4, CD-20, or CD-28 or human leukocyte antigen have been introduced (Blauwet and Cooper Jr 2010).

Acute myocarditis with viral etiology is the most common cause for myocarditis in Western Europe and North America (Cooper Jr 2009; Blauwet and Cooper Jr 2010; Sagar et al. 2011; Calabrese et al. 2010; Schultz et al. 2009; Yajima et al. 2009; Kühl and Schultheiss 2009). A study

Autoimmune Myocarditis and Pericarditis, Table 1 Proposed classification scheme for myocarditis (Modified from Sagar et al. 2011)

Classification scheme				
Cause	Histology	Immunohistology	Clinicopathological	Clinical
Viral	Eosinophilic	14 or more CD3+ or CD68+ cells/high power field	Fulminant	Acute heart failure
Enteroviruses (Coxsackie B)	Giant cell	Increased expression of leucocyte antigens (HLA-DR, etc.)	Acute	Syncope
	Granulomatous		Chronic active	Chest pain
Erythroviruses (Parvovirus B19)	Lymphocytic	Increased expression of adhesion molecules (ICAM1, etc.)	Chronic persistent	Myopericarditis
Adenoviruses				
Herpes viruses (Herpes simplex 6)				
Bacterial				
Borrelia burgdorferi,				
Corynebacterium Diphteriae				
Protozoal				
Tryponosoma cruzi				
Toxic				
Alcohol				
Radiations				
Chemicals				
Drugs				
Hypersensitivity				
Drugs				

on 172 cardiomyopathy patients (Kühl et al. 2005) in which polymerase chain reaction (PCR) had demonstrated viral genomes in the biopsy samples showed that the most common viruses were adenovirus, parvovirus B19 (although present in a large percentage of patients who do not have myocarditis (Yajima et al. 2009)), human herpesvirus 6, and enteroviruses like Coxsackie B. Other viruses often found to cause myocarditis are, for example, influenzavirus or Epstein-Barr virus.

The following model of viral infection of cardiac cells has been proposed: Both coxsackievirus and adenovirus use the same receptor, the coxsackievirus and adenovirus receptor (CAR), to infect a cardiac cell. In the absence of this receptor and co-receptor, the virus does not infect the cell. The high level of CAR expression in the heart at young ages might be one of the reasons for the apparently higher incidence of myocarditis in children. Viruses that overcome the innate immune system replicate and (in the mouse) lead to the production of viral proteins which can cause direct myocardial injury. Six to seven days after virus infection, the adaptive immune system is activated, triggering among others the infiltration of T lymphocytes in the heart (maximal infiltration 7–14 days after virus infection), leading to both the clearance of virus-infected myocytes and myocardial injury or necrosis (Blauwet and Cooper Jr 2010; Yajima et al. 2009; Kühl and Schultheiss 2009).

Besides viruses, other agents are known to cause myocarditis.

The spirochete *Borrelia burgdorferi*, trigger of Lyme disease, can cause acute and, rarely, chronic myocarditis, particularly in children (Cooper Jr 2009).

A protozoan agent to cause myocarditis is *Trypanosoma cruzi*, very common in Central

and South America. Here it may cause acute myocarditis or chronic myocardiopathy, supposedly through immune activation after infection (Aretz et al. 1987; Cooper Jr 2009; Sagar et al. 2011).

Other causes of myocarditis may be toxic chemicals such as hydrocarbons and arsenic. Many substances have reported to induce hypersensitivity reactions which lead to inflammation of the myocardium. Common medical agents to provoke similar reactions include ampicillin, clozapine, or sulfonamide drugs. Environmental agents implicated in myocarditis include lead, arsenic, or spider venom. Physical causes may be found in electrocution or radiation therapy. Immunological etiologies encompass a vast number of clinical syndromes, such as allergic reactions, rheumatoid arthritis, giant cell arteritis, rejection of graft after heart transplantation, and many more (Sagar et al. 2011).

An acute myocardial infarction-like syndrome with normal coronary arteries as well as heart failure, even with dilated left ventricle, have a good prognosis, while ventricular arrhythmias and high-degree heart block do not.

Symptoms

Various symptoms are associated with myocarditis, including fatigue, generalized body swelling, arthralgia, myalgia, orthopnea, tachypnea, and tachycardia – out of proportion to fever, palpitations, a "stabbing" pericardial chest pain which cannot be differentiated from acute coronary syndrome, cardiac dysrhythmias, circulatory impairment due to left ventricular failure, syncope, fulminant congestive heart failure, cardiogenic shock, sudden death, and many more. In the European Study of the Epidemiology and Treatment of Inflammatory Heart Disease (ESETCID), 3,055 patients with suspected myocarditis were screened for their various symptoms: 72 % showed dyspnea, 32 % had chest pain, and 18 % showed arrhythmias (Hufnagel et al. 2000). In pediatric patients the symptoms tend to be less specific, including poor appetite and sweating while feeding, fever, abdominal pain, respiratory distress, or chronic cough, which is often misinterpreted as asthma or pneumonia. In severe cases cyanosis may be noticed. In more than half of all cases, myocarditis follows a respiratory infection and patients report a history of fever, rash, joint pain, cough, or diarrhea, while symptoms specific for the heart usually arise during the subacute virus-clearing phase, approximately 2 weeks after viremia. Ventricular dysfunction due to a strong inflammation of the heart presents the classic symptoms, such as ascites and peripheral edema.

Auscultatory murmurs of mitral or tricuspid regurgitation may indicate ventricular dilation, while in advanced cases with an evolved dilated cardiomyopathy peripheral or pulmonary thromboembolism may be found. Lastly, the evolving inflammation may yield pericardial effusion of inflammatory infiltrate without tamponade and resulting pericardial and pleural friction rub (Schultz et al. 2009).

Pathophysiology

In fulminant myocarditis there is often evidence of viral RNA into patient's cells, suggesting cytotoxic necrosis without the formation of infiltrates. The progression in this case is exceptionally fast, and severe ventricular dysfunction may occur within 1 or 2 days. Macrophage activation and the resulting cytokine release lead to accumulation of a histologically visible interstitial infiltrate of mononucleated cells. Natural killer cells target damaged myocardial cells expressing viral proteins, helping to extend the process, which takes 4–14 days after viral infection, and prolonging the typical course of the disease (Cooper Jr 2009; Sagar et al. 2011; Schultz et al. 2009). Cytotoxic T lymphocytes will infiltrate the myocardium in later stages of the disease, triggering apoptotic cell death of such myocytes that present viral proteins on the cell surface. In many patients viral genomes are persistent in the myocardium. Overexpression of the cytokine tumor necrosis factor alpha (TNF-α) has been implicated in the progression of the disease and the development of irreversible heart failure (Calabrese et al. 2010; Schultz et al. 2009). When chronic myocarditis develops, the immune response itself leads to tissue destruction and remodelling, resulting in dilated

cardiomyopathy, in ventricular dysfunction, and ultimately in chronic heart failure. In case of acute myocarditis, the primary damage to the myocardium usually leads to myocardial cell death and thus liberation of a critical amount of cardiac self-antigens previously hidden to the immune system. In most patients with viral myocarditis, in fact, the pathogen is cleared and the immune system is downregulated with no further adverse effects. However, in a minority of patients, the virus is not cleared, resulting in persistent myocyte damage and myocardial inflammation due the immune response to cardiac autoantibodies (Blauwet and Cooper Jr 2010).

Diagnosis

Erythrocyte sedimentation rate, C-reactive protein concentration, or an increased level of antiviral antibody may help to diagnose acute myocarditis but they are nonspecific.

Sinus tachycardia, impairment of left ventricular systolic and diastolic function, compromised ejection fractions, conduction delays, and segmental wall motion abnormalities can be shown by electrocardiographic (ECG) examination. Changes include sinus tachycardia, ST-wave and T-wave abnormalities, and occasionally atrioventricular or bundle branch block. Associated with poor prognosis in acute myocarditis are widened QRS and Q waves (Cooper Jr 2009; Sagar et al. 2011; Kühl and Schultheiss 2009). Elevated rates of cardiac troponins or myosin are known markers for myocardial damage but are also unspecific.

Echocardiography helps to establish left ventricular (LV) and right ventricular (RV) function and to rule out other causes of heart failure. In myocarditis the usual findings are global hypokinesis with or without pericardial effusion (Cooper Jr 2009; Schultz et al. 2009).

A coronary catheterization will reveal normal coronary vessels and regional wall motion abnormalities with diminished ejection fraction and can help to exclude an ischemic origin of the patient's symptoms.

Diagnosis of myocardial viral infection and associated inflammatory process can only be confirmed by analysis of endomyocardial biopsies (EMB). This invasive procedure is performed in patients with unexplained acute-onset heart failure with hemodynamic compromise (Calabrese et al. 2010; Schultz et al. 2009; Kühl and Schultheiss 2009). Biopsies are examined histologically, often including immunostains for surface antigens such as anti-CD3, anti-CD4, anti-CD20, anti-CD28, anti-CD68, and antihuman leukocyte antigens. The interstitium may show abundant edema, focal destruction of myocytes, fibrosis, and infiltrates (Blauwet and Cooper Jr 2010; Schultz et al. 2009; Kühl and Schultheiss 2009). The introduction of amplification methods like PCR allows the detection of low copy viral genomes even from small biopsy samples. However, it is very important to use these molecular techniques in combination with other clinical and morphological techniques since the mere presence of viral nucleotide sequences does not imply a direct role of the virus in question in the pathogenesis of myocarditis. It must be remembered that only a positive PCR result is of diagnostic relevance, while a negative result for virus nucleotides does not exclude viral disease because the causative virus may not have been included in the analysis (Caforio et al. 2009; Schultz et al. 2009).

Noninvasive cardiac magnetic resonance imaging (MRI) may provide additional diagnostic value. With the unique potential for tissue characterization using T1- and T2-weighted images, cardiac MRI can evaluate three important markers of tissue injury: (i) intracellular and interstitial edema, (ii) hyperemia and capillary leakage, and (iii) necrosis and fibrosis (Sagar et al. 2011).

Treatment

Patients should receive standard heart failure care as outlined in the ACC/AHA/ESC and heart failure society guidelines.

Treatment trials of subpopulations of chronic viral-associated and nonviral myocarditis with biopsy-guided therapy are an area of active investigation. The finding of viral genomes on endomyocardial biopsy has been used to guide treatment in acute and chronic cardiomyopathy. In some but not all studies, the presence of viral

genomes was associated with subsequent worsening of heart function and the need for cardiac transplantation. Interferon beta (IFN-β) has been used successfully in patients with viral persistence in chronic, stable dilated cardiomyopathy. Viral clearance was achieved in all patients after antiviral treatment, with a significant increase in left ventricular function in the treatment group. Ongoing trials of antiviral treatment such as the use of IFN-β may lead to the use of specific antiviral treatment in the future. Conversely, patients with chronic dilated cardiomyopathy and no evidence of viral genome in the heart tissue may benefit from immunosuppressive therapy if preliminary results are confirmed in randomized, controlled trials. Immunosuppressive treatments might also prevent progression of some forms like the giant cell myocarditis by allowing control over the autoinflammatory process.

In patients whose condition deteriorates despite optimal medical management, mechanical circulatory support, such as ventricular assist devices or extracorporeal membrane oxygenation, as a bridge to transplantation or recovery, are suggested.

Autoimmune Myocarditis

Some autoimmune diseases, such as systemic lupus erythematosus, scleroderma, and vasculitides, may involve the myocardium. Interestingly, Frustaci (2002) state that serological detection of cardiac autoantibodies, HLA (human leukocyte antigen) profile, negative PCR studies for cardiotropic viruses, and responsiveness to a gluten-free diet and immunosuppressive therapy suggest the existence of an autoimmune disorder directed toward antigenic components of both the myocardium and small bowel celiac disease in their patients. Ventura et al. go even further in stating that the severity of associated autoimmune diseases in celiac disease patients can be influenced by a gluten-free diet (Ventura et al. 1999).

Autoimmunity is one of the main mechanisms in the pathogenesis linking myocarditis to dilated cardiomyopathy (DCM). Autoimmune myocarditis is thought to be precipitated by an initial infection with a pathogen, such as a virus. It may express surface epitopes which are highly similar to host epitopes found on the cell surface of cardiomyocytes, a process known as molecular mimicry. Some pathogens such as coxsackievirus B have epitopes immunologically similar to cardiac proteins. Animals developing autoimmune myocarditis after Coxsackievirus B3 infection show an abundance of antibodies to multiple cardiac antigens, for example, to epitopes of cardiac myosin. After having been activated by the virus, the immune system may attack these cardiac proteins.

In viral myocarditis the cardiomyocytes infected with a virus express HLA class I receptors with bound viral peptide antigens and effector CD8+ lymphocytes kill the infected cells inducing apoptosis. CD4+ helper T lymphocytes recognize antigens bound to HLA class II receptors on B cells. Upon activation they activate primed B cells to differentiate into memory B cells and effector plasma B cells which secrete antibodies. Virus-specific antibodies opsonize free virus particles which, once opsonized, form immune complexes which are then detected and destroyed by phagocytes.

Antibodies of both the IgM and IgG classes can activate the classical complement cascade leading to the destruction of uninfected cells and the release of large quantities of intracellular and hence immunologically protected antigens that are taken up by macrophages. These will present self-peptides to autoreactive CD4+ T helper cells. The result is a failure of tolerance. Antibodies directed against intracellular host antigens, including anti-nucleic acid, anti-myosin, anti-troponin I, and anti-actin antibodies are found in the bloodstream of patients suffering from autoimmune myocarditis.

Antibodies against cardiac troponin I (TnI) lead to severe inflammation and fibrosis in the myocardium in a murine model leading to cardiac dilatation and reduced survival (Kühl and Schultheiss 2011; Rose 2009; Kaya et al. 2010).

The removal of autoantibodies might positively influence the progression of the disease. In pilot studies various antibodies, including the

cardiodepressive autoantibodies of the IgG class, were removed from patient's blood by immunoadsorption and, in order to prevent infections, replaced by 0.5 mg/kg KG polyclonal IgG with promising results. About 60 % of the treated patients experienced improvement of their condition (Rose 2009), and later studies showed that even a single treatment could lead to a lasting positive effect (Kühl and Schultheiss 2011; Rose 2009).

A study conducted by Watanabe (2001) indicated that delivery of plasmid DNA expressing IL-10, an anti-inflammatory cytokine produced mainly by monocytes and lymphocytes, by electroporation provides marked cardioprotection in a rat model of autoimmune myocarditis. IL-10 downregulates the expression of Th1 cytokines and MHC class II antigens and costimulatory molecules on macrophages while it enhances B-cell proliferation, survival, and antibody production.

Idiopathic giant cell myocarditis (GCM) is an autoimmune form of myocarditis histologically defined by the presence of multinucleated giant cells, a lymphocytic inflammatory infiltrate, and myocyte necrosis. Often it is fatal except when mechanical support or heart transplantation is performed (Blauwet and Cooper Jr 2010). Immunosuppressive treatment proved to delay fatality of GCM and improve long-term survival, while in many other forms of myocarditis, this treatment had mainly disappointing results (Blauwet and Cooper Jr 2010), although it was hypothesized that GCM is a heterogeneous disease with one form that can recover without immunosuppression and other forms that respond to immunosuppressive therapy (Murashita et al. 2006). Immunosuppressive therapy is also indicated in refractory cardiac insufficiency, after GCM, granulomatous myocarditis, or eosinophilic necrotizing myocarditis has been histologically confirmed (Cooper Jr 2009; Kühl and Schultheiss 2011).

These are rare diseases (they make up only about 10 % of acute myocarditis cases), but they have been shown to be successfully treated by immunosuppressive measures, when therapy was started early. Untreated they are coupled with a high mortality rate.

Pericarditis

The pericardium is a double-layered sac of connective tissue covering the outer surface of the heart. It is filled with a small, physiological amount of fluid which prevents the two layers from rubbing directly on each other causing friction and which augments in case of inflammation. It anchors the heart in the chest wall and shields it from infection. It also prevents the heart from overexpanding when blood volume increases. Pericarditis is an inflammation of the pericardium, often characterized by chest pain.

Pericarditis can be classified according to the composition of the inflammatory exudate found between the layers of the pericardium (pericarditis exudativa) into serous pericarditis, fibrinous p., purulent p., hemorrhagic p., and tuberculous p. as forms of acute pericarditis and *concretio pericardii* (adhesive pericarditis), *accretio pericardii*, and *constrictio pericardii* (constrictive pericarditis) as forms of chronic pericarditis. In *concretio pericardii*, the epi- and pericardium become joined by a stringlike growth, while in *accretio pericardii* the pericardium becomes joined to the surrounding mediastinum, making adjustments of the heart's position in response to movement almost impossible. In *constrictio pericardii* the surface of the pericardial sac becomes obliterated by, for example, calcifications.

In pericarditis sicca there is no significant exudate detected. Pericarditis fibrinosa is the form usually present at the beginning with inflammatory infiltrates deposit between the two layers of the membrane. In p. exudativa there is typically less pain than in p. sicca and auscultation reveals diminished friction rub. In most cases pericarditis exudativa is caused by a viral or other infection or an immunological condition. The amount of exudate can limit the function of the heart to the point of cardiac tamponade and circulatory impairment and, in extreme cases, can lead to a shock. The heart may be compressed by the constrictive process, which may cause blood to back up into the lungs, abdomen, and legs causing edema.

Apart from pericardial effusion, later on the result is fibrosis and calcifications can be found, both of which significantly hinder the function of

the heart. If acute pericarditis lasts several months, it becomes a chronic form. Almost always the layers of myocardium under the inflamed tissue are influenced to some degree by the pericardial damage; in these cases a process called perimyocarditis is in effect. When endocardium is also involved, the process is called pancarditis. Pericarditis is likely to recur even after it was successfully treated.

Symptoms

Often pericarditis is characterized by intense substernal, retrosternal, or left precordial pleuritic chest pain which worsens in supine position or during cough, swallowing, or inspiration, while it does not worsen upon exertion and it eases up in sitting positions or when the patient leans forward. In this it differs from the pain experienced in angina pectoris. The pain may irradiate to the trapezius ridge and the left or both shoulders.

Cough, fever, fatigue, palpitations, tachypnea, diaphoresis, or syncope may be noticed, as well as mild dysrhythmias, pulsus paradoxus from a possible cardiac tamponade, distant heart sounds, jugular vein distention, and anxiety. Some of the symptoms are proportional to the amount of exudate present.

In chronic forms lethargy, cough, and respiratory distress may be noticed, as well as distant heart sounds upon auscultation and equilibration of all the diastolic blood pressures on cardiac catheterization due to the constriction of the pericardium. Other symptoms may be jugular vein distension and pulsus paradoxus. Chronic forms are usually characterized by absence of chest pain. Severe cases can lead to hypotension and hence water retention and edema of the lower extremities, liver, and stomach.

Diagnosis

Because of the similarity of the pain experienced by patients to the pain of a myocardial infarction, pericarditis can be misdiagnosed when solely based on clinical data.

Isolation of viruses causing pericarditis using PCR analysis is highly difficult due to the delay between the initial pericardial infection and the clinical manifestation of pericarditis, as well as the limited number of virus genomes which can be screened. PCR analysis allows for precise etiological characterization and reduces the number of cases of idiopathic pericarditis greatly; however PCR analysis is usually reserved to recurrent cases, as the disease resolves quite often spontaneously (Calabrese et al. 2010).

Often alterations of the ECG can be observed, such as repolarization troubles with ST-segment anomalies. By echocardiography even small amounts of exudate, pus, or pericardial thickening can be detected. Transesophageal or transthoracic echocardiography is often used to diagnose cardiac tamponade. Upon auscultation a rubbing friction sound can be distinguished; this sound usually diminishes or vanishes in pericarditis exudativa.

X-ray of the thorax may reveal only substantial effusions, cardiomegaly, and/or heart congestion of the lungs.

Pericardiocentesis, while relieving the heart of some of the pressure from the high amount of exudate, may also allow detecting bacterial infections or cancerous cells. Association of pain typical for pericarditis and a heightened amount of urea acid or creatinine in the bloodstream may suggest uremic pericarditis.

Cardiac MRI and CT scans may reveal excess fluid in the pericardium, evidence of inflammation, and pericardial thickening, while cardiac catheterization will provide hemodynamic information.

Causes

The causes of pericarditis can be separated into infectious and noninfectious although the most frequently reported cause of acute pericarditis is idiopathic. Presumably, the latter is mostly of viral origin. Viral pericarditis is mainly caused by the same viruses that cause myocarditis (see above), particularly enteroviruses, adenoviruses, and influenza viruses (Calabrese et al. 2010). Others causes of pericarditis include bacterial or fungal infections – *Mycobacterium tuberculosis* being a common cause in developing nations, a condition with almost 85 % mortality rate within the first 6 months (Calabrese et al. 2010). Uremic pericarditis, Dressler's or

post-infarct pericarditis epistenocardica, kidney failure, and autoimmune diseases like systemic lupus erythematosus, systemic sclerosis, rheumatoid fever, rheumatoid arthritis, sarcoidosis, or Sjogren syndrome also should be mentioned. Pericarditis may result from genetic diseases such as Familial Mediterranean fever, as well as from associated myocarditis, neoplasias, hypothyroidism, immune deficiencies, radiation therapy, traumas of the thorax, and surgery. Rarely, it results from allergic reactions or side effects of pharmaceuticals.

Treatment

The basic treatment for pericarditis is limited to bed rest under strict ECG control and nonsteroidal anti-inflammatory drugs. More specific treatments depend on the causes and symptoms. Antibiotics or antimycotics may be needed in case of other infectious causes. Corticosteroids are reserved for rare cases and are seldom required, except in the case of certain systemic autoimmune diseases. Diuretics may be used in constrictive pericarditis to control fluid retention. Severe cases may require pericardiocentesis or surgery. When pericardiocentesis is ineffective, a pericardial window may be performed. Pericarditis constrictiva calcarea can in many cases be treated by pericardectomy.

Autoimmune Pericarditis

For a long time researchers have been discussing whether recurrent episodes of pericarditis could be attributed to autoimmune processes. To correctly diagnose autoimmune pericarditis, Shabetai (2005) advises that antisarcolemmal antibodies should be searched for, PCR for cardiotropic viruses and other infectious agents should be proven to be negative, and IgM against these agents should not be detectable.

Circumstantial evidence for pericarditis to be an autoimmune process is divided into 6 subgroups by Rose (2010):

(I) An increased number of pro-inflammatory cells and cytokines in the pericardial tissues and fluid
(II) Antibodies against pericardial tissue in serum and pericardial fluid
(III) Exclusion of the presence of an infectious agent by PCR, and no antibodies to an infectious agent in the serum
(IV) Weak but discernable statistical association with a particular HLA haplotype
(V) A slight but consistent male bias
(VI) Pericarditis found in association with other autoimmune diseases

Rose underlines, however, that it is still uncertain whether autoimmunity has a causative role or is a consequence of the inflammatory process.

The similarities between idiopathic recurrent pericarditis and autoinflammatory diseases, together with the marked efficacy of anakinra, a drug developed to treat rheumatoid arthritis, in their patients, led Picco et al. to suppose that at least a subset of patients diagnosed as having idiopathic recurrent pericarditis could be affected by a autoinflammatory disease, possibly related to gene mutations leading to dysregulation of IL-1β production and secretion (Picco et al. 2009).

Dressler's Syndrome

Dressler's syndrome, also known as post-myocardial infarction syndrome, post-cardiac injury syndrome, or postpericardiotomy syndrome, develops between the second and tenth week after a myocardial infarction, traumatic injury, and heart surgery or rarely after percutaneous procedures like stent or pacemaker implantations. It is thought to be the result of an autoimmune response to myocardial neo-antigens following damage to the heart tissue. It is usually self-limiting and does only very seldomly lead to cardiac tamponade. Showing most of the symptoms of other forms of pericarditis, it may rarely lead to cardiac tamponade, pleuritis, pulmonary infiltration, or pericarditis. It tends to subside within a few weeks but may recur. It is possible to find pleuritis, sometimes with pleural effusions, in some patients. It needs to be distinguished from pulmonary embolism which also causes pleuritic chest pain and often occurs in hospitalized patients recovering from heart injury. The treatment of Dressler's syndrome is concurrent with that of other forms

of pericarditis. Treatment usually concentrates on medical agents which decrease the inflammatory response such as nonsteroidal anti-inflammatory drugs or with corticosteroids. However corticosteroids are reserved for rare cases and are seldom required. Inpatient care is usually necessary only in cases which indicate a possible tamponade. A rare but fatal complication of Dressler's syndrome is the occlusion of coronary artery bypass grafts.

Cross-References

- ▶ Autoimmune Heart Disease: Animal Models
- ▶ Autoinflammatory Diseases
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Viral Myocarditis

References

Aretz HT, et al. Myocarditis: a histopathologic definition and classification. Am J Cardiovasc Pathol. 1987;1:3–14.

Blauwet LA, Cooper Jr LT. Myocarditis. Prog Cardiovasc Dis. 2010;52:274–88.

Caforio AL, Tona F, Vinci A, Calabrese F, Ramondo A, Cacciavillani L, Corbetti F, Leoni L, Thiene G, Iliceto S, Angelini A. Acute biopsy-proven lymphocytic myocarditis mimicking Takotsubo cardiomyopathy. Eur J Heart Fail. 2009;11(4):428–31. doi: 10.1093/eurjhf/hfp008. Epub 2009 Feb 3.

Calabrese F, Carturan E, Thiene G. Cardiac infections: focus on molecular diagnosis. Cardiovasc Pathol. 2010;19:171–82.

Cooper Jr LT. Myocarditis. N Engl J Med. 2009;360:1526–38.

Frustaci A. Celiac disease associated with autoimmune myocarditis. Circulation. 2002;105:2611–8.

Hufnagel G, et al. The European study of epidemiology and treatment of cardiac inflammatory diseases (ESETCID) first epidemiological results. Herz. 2000;25:279–85.

Kaya Z, et al. Cardiac troponins and autoimmunity: their role in the pathogenesis of myocarditis and of heart failure. Clin Immunol. 2010;134(1):80–8.

Kühl U, Schultheiss HP. Spezifische therapie der inflammatorischen kardiomyopathie und viralen herzerkrankungen. J Kardiol. 2011;18(1–2):16–23.

Kühl U, Schultheiss HP. Viral myocarditis diagnosis aetiology and management. Drugs. 2009;69(10):1287–302.

Kühl U, Schultheiss HP, et al. Viral persistence in the myocardium is associated with progressive cardiac dysfunction. Circulation. 2005;122(13):1965–70.

Murashita T, et al. A case of giant cell myocarditis: bridge to recovery by long-term mechanical circulatory support without immunosuppressive therapy. J Thorac Cardiovasc Surg. 2006;132:432–3.

Picco P, et al. Successful treatment of idiopathic recurrent pericarditis in children with interleukin-1beta receptor antagonist (anakinra) an unrecognized autoinflammatory disease? Arthritis Rheum. 2009;60(1):264–8.

Rose NR. Myocarditis: infection versus autoimmunity. J Clin Immunol. 2009;29:730–7.

Rose NR. Recurrent pericarditis: an autoimmune disease? Heart. 2010;96(10):734–5.

Sagar S, Liu PP, Cooper Jr LT. Myocarditis. Lancet. 2011;379:738–47.

Schultz JC, et al. Diagnosis and treatment of viral myocarditis. Mayo Clin Proc. 2009;84:1001–9.

Shabetai R. Recurrent pericarditis: recent advances and remaining questions. Circulation. 2005;112:1921–3.

Ventura A, Magazzu G, Greco L. Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease: SIGEP study group for autoimmune disorders in celiac disease. Gastroenterology. 1999;117:297–303.

Watanabe K. Protection against autoimmune myocarditis by gene transfer of interleukin-10. Circulation. 2001;104:1098–100.

Yajima T, et al. Viral myocarditis from the perspective of the virus. Circulation. 2009;119:2615–24.

Autoimmunity and Inflammation in Diabetic Nephropathy

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Synonyms

Diabetic kidney disease; Diabetic nephropathy

Definition

This entry reviews current understanding of the contribution of autoimmunity and inflammation to the development and progression of diabetic kidney disease.

Introduction

Inflammatory autoimmune beta cell destruction in the pancreas (insulitis) is characteristic of type 1 diabetes, whereas type 2 diabetes is a complex, polygenic disease that involves combinations of insulin resistance and defects in insulin secretion. Although type 1 diabetes results from an immune-mediated inflammatory process, the pathogenesis of diabetic nephropathy, one of the chronic microvascular complications of diabetes, is generally considered to be multifactorial. Although associated with inflammation at various stages (see below), the genesis of the specific lesions of diabetic nephropathy is not thought to be via an autoimmune mechanism.

Glycemic control is strongly associated with development and progression of diabetic nephropathy. In the Diabetes Control and Complications Trial (DCCT) study, the risk of microalbuminuria increased geometrically with the increasing hemoglobin A_{1c} (A_{1c}) levels during the study (The Diabetes Control and Complications Trial Research Group 1993). Moreover, the DCCT (type 1 diabetes) and the United Kingdom Prospective Diabetes Study (UKPDS; type 2 diabetes) demonstrated the benefit of strict glycemic control on the development and progression of microalbuminuria and overt proteinuria, as predictors and indicators of diabetic nephropathy (The Diabetes Control and Complications Trial Research Group 1993; UK Prospective Diabetes Study (UKPDS) Group 1998). In type 1 diabetic patients who had a kidney transplant, diabetic nephropathy lesions, manifesting as increased glomerular basement membrane width (GBM) and mesangial and mesangial matrix fractional volumes, developed at rates similar to those seen in the native kidneys of type 1 diabetic patients. This occurred despite immunosuppressive therapy in the diabetic recipients beginning when the normal kidney was first placed into a diabetic environment (Chang et al. 2008). Furthermore, diabetic nephropathy lesions can be reversed after 10 years of normoglycemia by pancreas transplantation (Fioretto et al. 1998) with the same immunosuppression that allowed lesions to develop in the renal transplant recipient with type 1 diabetes. Finally, diabetic nephropathy lesions do not develop in nondiabetic members of identical twin pairs discordant for type 1 diabetes, despite presumably equal genetic propensity to autoimmune endocrinopathies (Steffes et al. 1985). However, although these studies all indicate the critical requirement and importance of glycemia to the development and progression of diabetic nephropathy, the above arguments do not exclude the contribution of autoimmunity and/or inflammatory processes in the genesis or progression of diabetic nephropathy lesions.

Interestingly, the presence of T-lymphocytes was identified in the region of the juxtaglomerular apparatus (JGA) (where renin is produced) in 60 % of type 1 diabetic patients with and without abnormal urinary albumin excretion (Paulsen et al. 1994). Moriya et al. (2004) found that JGA T-cell positive patients had shorter duration of diabetes (mean 6.7 years for T-cell positive vs. 9.2 years for T-cell negative patients, $p = 0.011$) and lower urinary albumin excretion but similar A_{1c} values compared to T-cell negative patients (Moriya et al. 2004). GBM width and mesangial fractional volumes were similar in the two groups, but filtration surface per glomerulus was greater in the 37 T-cell positive vs. 38 T-cell negative young type 1 diabetic research patients (Moriya et al. 2004). This inflammatory process was presumed to represent an autoimmune process, part of the polyendocrinopathy which is highly associated with the autoimmune β-cell destruction leading to type 1 diabetes (Moriya et al. 2004). Reduction in glomerular filtration surface is an important component for the loss of glomerular filtration function in diabetic nephropathy. Thus, the possibility exists that this presumed JGA autoimmune process, perhaps through reduction in local renin production, may, in fact, be beneficial.

In patients with type 1 diabetes, there is markedly increased localization of plasma proteins mainly albumin and immunoglobulin (IgG) in extracellular basement membranes, including glomerular and tubular basement membrane (GBM, TBM), and Bowman's capsule (Michael and Brown 1981). This occurs irrespective of the severity of the underlying diabetic nephropathy

lesions but has the appearance of greater intensity and extent when these basement membranes are thickened. Binding of plasma proteins to basement membrane is charge-selective since the IgG found in these renal basement membranes is restricted to the IgG4 subclass, with its relatively low isoelectric point, in contrast to anti-GBM diseases, where all IgG subclasses are bound to GBM (Melvin et al. 1984). Nevertheless, the mechanism whereby there is increased binding of anionic proteins to the presumably positively charged renal basement membrane in diabetic nephropathy is still unclear. The importance is that diabetes should not be confused with anti-GBM antibody diseases, a distinction greatly facilitated by the diagnosis of diabetes, the increased albumin localization on renal extracellular basement membranes, and the absence or paucity of glomerular inflammatory cells in diabetic patients.

Plasma proteins are not only observed in renal basement membrane but also in glomerular arteriolar hyalinosis lesions appearing by light microscopy as periodic acid-Schiff (PAS) stained-positive opaque ground glass. These afferent and efferent arteriolar hyaline lesions can be found only after a few years of diabetes (Harris et al. 1991). Accumulations beneath the internal elastic lamina can extend into and replace arteriolar smooth muscle cells and ultimately occlude the vascular lumina. When advanced they are associated with increased numbers of globally sclerotic glomeruli (Harris et al. 1991). Immunohistochemical studies of these exudative lesions have identified the presence of albumin, immunoglobulins, complement, fibrinogen, and other plasma proteins (Falk et al. 1983). Identical hyaline accumulations can occur as subendothelial deposits in glomerular capillaries which, through a process of adhesion and extraction (personal observations), can evolve to capsular drops, that is, hyaline deposits along Bowman's membrane. The plasma proteins localized in these exudative lesions can fix heterologous guinea pig complement, indicating their inflammatory potential (Burkholder 1965). However, these lesions are rarely associated with visible tissue inflammation (personal observations). Additionally, immunofluorescent studies using antibodies to C5–9 complement components of the membrane attack complex (MAC) revealed extensive localization of MAC in the mesangium, juxtaglomerular area, Bowman's capsule, afferent and efferent arterioles, and TBM in patients with advanced diabetic nephropathy as well as in other non-nephritic renal diseases such as hypertension, amyloidosis, and obstructive uropathy (Falk et al. 1983). Thus, these findings are not specific for diabetic nephropathy, more likely represent inflammation consequences of advanced renal injury, but may be very important in mechanisms of advanced renal disease progression. Thus, the deposition of immunoglobulins and complement components from the classical and alternative pathway is present in sclerosed glomeruli in end-stage renal disease specimens regardless of the cause the kidney failure.

Several additional studies have also suggested the involvement of complement activation in the pathogenesis of diabetic nephropathy. Mannose-binding lectin or MBL, which is primarily synthesized in the liver and specifically binds to the glycosylated surface of microorganisms, can activate the complement pathway through MBL-associated serine proteases. Elevated circulating MBL levels were found in patients with type 1 diabetes and positively correlated with urinary albumin excretion (Hovind et al. 2005). Moreover, serum MBL levels were significantly higher in patients with nephropathy compared to normoalbuminuric patients (Hovind et al. 2005), and higher MBL levels in early course of type 1 diabetes are associated with later development of persistent albuminuria (Hovind et al. 2005). Although the deposition of MBL in the diabetic kidney has not yet been evaluated, MBL-mediated complement activation could contribute to diabetic microvascular complications. However, the MBL-mediated complement pathway is not specific to diabetes. Glomerular deposition of MBL and MBL-associated serine protease are also found in patients with Henoch-Schönlein purpura and IgA nephropathy. Whether complement activation, particularly through the MBL pathway, plays a specific role in the pathogenesis of diabetic kidney complications or contributes as a common pathway to nonspecific inflammation in diabetes needs to be elucidated.

It is important to understand that although direct tissue pathologic findings of renal inflammatory processes are limited at the earlier stages of the development of classical diabetic nephropathy lesions, the nature and patterns of the lesions change as the disease progresses. Thus, when proteinuria appears, which in type 1 diabetes is virtually always associated with advanced mesangial expansion, new pathologic changes emerge which are rarely seen earlier in the disease. These include tuft adhesions and focal segmental glomerulosclerosis lesions at or close to the glomerular-tubular junction, along with varying degrees of severity of damage to the early portion of the proximal tubule (Najafian et al. 2006). Associated with tubular damage, there is increased interstitial fibrosis and influx of inflammatory cells into the interstitium and tubules (Najafian et al. 2006). In more advanced cases, with elevated serum creatinine levels above 1.5 mg dl, these inflammatory changes become increasingly severe and are, together with interstitial fibrosis, associated with further glomerular filtration rate decline and progression to end-stage renal disease (Bohle et al. 1987). However, these changes are not unique to diabetic nephropathy and appear to represent final common pathways of progressive renal injury as end-stage renal disease is approached in a wide variety of renal disorders. This inflammation may be the downstream consequence of proteinuria per se and/or the scavenger tissue responses to advanced nephron cellular injury and death (Abbate et al. 2006).

There are, however, a number of lines of evidence supporting the view that mediators of inflammation may become activated earlier in the course of diabetic nephropathy. A number of studies have connected diabetic nephropathy status and risk with inflammatory cytokines. Thus, Dalla Vestra et al. (2005) in studies of research biopsies in type 2 diabetic patients found that increased GBM width was associated with greater levels of serum amyloid protein, C-reactive protein, and interleukin-6, and GBM width was directly correlated with fibrinogen levels ($r = 0.33$, $p < 0.002$) and interleukin-6 ($r = 0.25$, $p < 0.05$, respectively) (Dalla Vestra et al. 2005). Interleukin-18 was overexpressed in tubular cells in most biopsies of type 2 diabetic patients with overt nephropathy, while this was rarely the case in minimal change nephrotic syndrome patients, suggesting that this was not the consequence of proteinuria per se (Miyauchi et al. 2008). Nakamura et al. (2005) in studies of type 2 diabetic patients found direct correlations between albumin excretion rate and serum and urine interleukin-18 levels ($r = 0.53$, $p < 0.001$ and $r = 0.31$, $p = 0.005$, respectively) (Nakamura et al. 2005).

Importantly, in a study of 249 Japanese type 2 diabetic patients, elevated IL-18 levels strongly predicted transition from normoalbuminuria to microalbuminuria [(OR 3.695 % (I 1.2–10.4) for serum interleukin-18 levels above the median] (Araki et al. 2007). Moreover, in patients with interleukin-18 serum levels above the median, the annual rate of decline of glomerular filtration rate (−2.1 [interquartile range −0.5–3.0] ml/min/1.73 m^2/year) was greater than in patients with lower interleukin-18 serum values (−1.1 [interquartile range −0.2 −2.9] ml/min/1.73 m^2/year), $p = 0.036$ (Araki et al. 2007).

More recently, two important papers were published which further solidified the importance of inflammatory cytokine variables as predictors of diabetic nephropathy risk and progression. In 625 type 1 diabetic patients with normal estimated glomerular filtration rate and high normal albumin excretion rate ($n = 146$) or microalbuminuria ($n = 482$), the risk of the subsequent development of estimated glomerular filtration rate values <60 ml/min/1.73 m^2 was strongly related with the baseline quintile distribution of the levels of circulating tumor necrosis factor (TNF) receptors 1 and 2 obtained 5–12 years earlier (Gohda et al. 2012). A second study from this same group described TNF receptors 1 and 2 levels in 410 (85 % Caucasian) type 2 diabetic patients with chronic kidney disease stages ≤3 (Niewczas et al. 2012). On follow-up 8–12 years later, they were categorized as alive, end-stage renal disease, or deceased without end-stage renal disease. The end-stage renal disease group had much higher baseline albumin excretion rate and lower estimated glomerular

filtration rate values than the other groups (Niewczas et al. 2012). Markers of endothelial function and cytokines, including intercellular adhesion molecule-1, vascular intercellular adhesion molecule-1, plasminogen activator inhibitor-1, interleukin-6, and C-reactive protein, were similar in all groups, while TNF pathway markers (free TNFα, total TNFα, and TNF receptors 1 and 2) were highest in the end-stage renal disease group and intermediate in the deceased without end-stage renal disease group. TNF receptors 1 and 2 were the strongest predictors. These findings remained statistically significant after controlling for baseline glycemia, albumin excretion rate, and estimated glomerular filtration rate (Niewczas et al. 2012).

Other renal diseases can develop or be superimposed on diabetic nephropathy in patients with types 1 or 2 diabetes. Immunologic-related renal diseases such as minimal change nephrotic syndrome and membranous nephropathy may occur more commonly in the patient with type 1 diabetes than in nondiabetic patient. Less than 1 % of type 1 diabetic patients who had diabetes more than 10 years and fewer than 4 % of those who had proteinuria were found to have renal disorders other than diabetic nephropathy when the biopsies were performed for research purposes (Mauer M, personal observation). Moreover, there are no reports suggesting that autoimmune renal diseases, such as anti-GBM antibody or lupus nephrites, are more common in people with vs. without diabetes. Different patterns of renal injury in diabetic patients biopsied for clinical indications are substantially influenced by the criteria or indication using for biopsy. Diabetic glomerulosclerosis was observed more commonly in patients in institutions where there was less restrictive renal biopsy indication, whereas the incidence of other glomerulonephritis superimposed or without diabetic kidney disease was remarkably higher in patients at institutions with more restrictive biopsy criteria which limited biopsy to patients with more atypical clinical presentations (Mazzucco et al. 2002). Thus, patients with atypical courses, for example, proteinuric type 1 diabetic patients whose diabetes duration is less than 10 years or proteinuric type 2 diabetic patients with albuminuria and/or reduced glomerular filtration rate but without retinopathy, should be carefully evaluated for renal disorders other than diabetes, and renal biopsy should be considered in these cases.

Conclusion

In summary, although evidence that autoimmune renal injury is important in the genesis and progression of diabetic nephropathy is not compelling, there are strong indicators that inflammatory processes are involved in the genesis and progression of diabetic nephropathy in patients with type 1 and type 2 diabetes. Moreover, the progression of many renal diseases from stages of well-established parenchymal damage to end-stage renal disease may include elements of autoimmunity processes (Bohle et al. 1987) that remain to be fully explored. It is not yet clear if targeting such inflammatory processes may be therapeutically beneficial in diabetic nephropathy.

Cross-References

- ▶ Anti-glomerular Basement Membrane Disease
- ▶ Atherosclerosis and Cytokines
- ▶ Macrophages, Oxidative Stress, and Atherosclerosis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Complement Regulation in the Kidney
- ▶ Fibrosis
- ▶ NF-κB
- ▶ TGF-β

References

Abbate M, Zoja C, Remuzzi G. How does proteinuria cause progressive renal damage? J Am Soc Nephrol. 2006;17(11):2974–84.

Araki S, Haneda M, Koya D, Sugimoto T, Isshiki K, Chin-Kanasaki M, Uzu T, Kashiwagi A. Predictive impact of elevated serum level of IL-18 for early renal dysfunction in type 2 diabetes: an observational follow-up study. Diabetologia. 2007;50(4):867–73.

Bohle A, Mackensen-Haen S, Gise HV. Significance of tubulointerstitial changes in the renal cortex for the excretory function and concentration ability of the kidney: a morphometric contribution. Am J Nephrol. 1987;7:421–33.

Burkholder PM. Immunohistopathologic study of localized plasma proteins and fixation of guinea pig complement in renal lesions of diabetic glomerulosclerosis. Diabetes. 1965;14(12):755–70.

Chang S, Caramori ML, Moriya R, Mauer M. Having one kidney does not accelerate the rate of development of diabetic nephropathy lesions in type 1 diabetic patients. Diabetes. 2008;57:1707–11.

Dalla Vestra M, Mussap M, Gallina P, Bruseghin M, Cernigoi AM, Saller A, Plebani M, Fioretto P. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. J Am Soc Nephrol. 2005;16(Suppl 1):S78–82.

Falk RJ, Dalmasso AP, Kim Y, Tsai CH, Scheinman JI, Gewurz H, Michael AF. Neoantigen of the polymerized ninth component of complement. Characterization of a monoclonal antibody and immunohistochemical localization in renal disease. J Clin Invest. 1983;72(2):560–673.

Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med. 1998;339:69–75.

Gohda T, Niewczas MA, Ficociello LH, Walker WH, Skupien J, Rosetti F, Cullere X, Johnson AC, Crabtree G, Smiles AM, Mayadas TN, Warram JH, Krolewski AS. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 2 diabetes. J Am Soc Nephrol. 2012;23:516–24.

Harris RD, Steffes MW, Bilous RW, Sutherland DE, Mauer SM. Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin dependent diabetes. Kidney Int. 1991;40(1):107–14.

Hovind P, Hansen TK, Tarnow L, Thiel S, Steffensen R, Flyvbjerg A, Parving HH. Mannose-binding lectin as a predictor of microalbuminuria in type 1 diabetes: an inception cohort study. Diabetes. 2005;54(5):1523–7.

Mazzucco G, Bertani T, Fortunato M, Bernardi M, Leutner M, Boldorini R, Monga G. Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. Am J Kidney Dis. 2002;39(4):713–20.

Melvin T, Kim Y, Michael AF. Selective binding of IgG4 and other negatively-charged plasma proteins in normal and diabetic human kidneys. Am J Pathol. 1984;115:443–6.

Michael AF, Brown DM. Increased concentration of albumin in kidney basement membranes in diabetes mellitus. Diabetes. 1981;30(10):843–946.

Miyauchi K, Takiyama Y, Honjyo J, Tateno M, Haneda M. Upregulated IL-18 expression in type 2 diabetic subjects with nephropathy: TGF-beta 1 enhanced IL-18 expression in human renal proximal tubular epithelial cells. Diabetes Res Clin Pract. 2008;83(2):190–9.

Moriya R, Manivel JC, Mauer M, for the International Diabetic Nephropathy Study Group (IDNSG). Juxtaglomerular apparatus T-cell infiltration affects glomerular structure in Type 1 diabetic patients. Diabetologia. 2004;47:82–8.

Najafian B, Crosson JT, Kim Y, Mauer M. Glomerulotubular junction abnormalities are associated with proteinuria in type 1 diabetes. J Am Soc Nephrol. 2006;17:S53–60.

Nakamura A, Shikata K, Hiramatsu M, Nakatou T, Kitamura T, Wada J, Itoshima T, Makino H. Serum interleukin-18 levels are associated with nephropathy and atherosclerosis in Japanese patients with type 2 diabetes. Diabetes Care. 2005;28(12):2890–5.

Niewczas MA, Gohda T, Skupien J, Smiles AM, Walker WH, Rosetti F, Cullere X, Eckfeldt JH, Doria A, Mayadas TN, Warram JH, Krolewski AS. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. J Am Soc Nephrol. 2012;23(3):507–15. Epub 2012 Jan 19.

Paulsen EP, Burke BA, Vernier RL, Mallare MJ, Innes Jr DJ, Sturgill BC. Juxtaglomerular body abnormalities in youth-onset diabetic subjects. Kidney Int. 1994;45(4):1132–9.

Steffes MW, Sutherland DER, Goetz FC, Rich SS, Mauer SM. Studies of kidney and muscle biopsy specimens from identical twins discordant for Type I diabetes mellitus. N Engl J Med. 1985;312:1282–7.

The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977–86.

UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352(9131):837–53.

Autoinflammatory Diseases

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Synonyms

Autoinflammatory disorders; Hereditary periodic fever syndromes; Hereditary recurrent fever syndromes

Definition

Autoinflammatory diseases were originally described as a group of hereditary disorders characterized by recurrent, seemingly unprovoked inflammatory episodes without involvement of the adaptive immune response, i.e., absence of high-titer pathogenic autoantibodies or self-antigen-specific T lymphocytes (McDermott et al. 1999).

Its definition has recently been updated to encompass a larger group of inflammatory disorders mediated predominantly by the innate immune system and associated with a significant host predisposition, either as a Mendelian or a complex trait (Kastner et al. 2010). This definition does not rule out the contribution of adaptive immune response to the inflammatory process, but it aims to distinguish autoinflammatory diseases from autoimmune disorders, which are thought to develop through the effector responses of T and/or B cells directed against self-antigens.

Autoinflammatory Features

The typical features of autoinflammatory disorders include:

- Recurrent and self-limited episodes of inflammation
- Varying degrees of fever during attacks
- Localized inflammatory manifestations, including peritonitis, pleuritis, arthritis, skin rash, intraocular inflammation, and/or neurologic findings
- Inflammatory attacks lasting several hours to weeks (depending on the underlying disease)
- Spontaneous resolution of the clinical findings and elevated inflammatory markers after each acute episode

Patients are usually normal between attacks, but some manifestations may be persistent. Although episodes of inflammation are typical, recurrence rates may change without a clear periodicity. With the identification of underlying pathogenic mechanisms such as inflammasome-associated inflammation, several other manifestations, such as vasculitic rash, vitiligo, or lipodystrophy, have been included into the autoinflammatory spectrum.

Manifestations and Differential Diagnosis

Distinctive features of the most common hereditary autoinflammatory disorders are summarized in Table 1. Familial Mediterranean fever (FMF), the prototypical and most common autoinflammatory disorder, is characterized by recurrent attacks of fever and serosal, synovial, or cutaneous inflammation lasting 12–72 h. Abdominal pain resulting from peritonitis presents as an "acute abdomen" and typically resolves within a few days. FMF usually starts during childhood, but milder or atypical forms can be seen after the age of 40. It usually demonstrates an autosomal recessive inheritance pattern and is quite commonly seen in patients with Jewish, Armenian, Turkish, Arab, or other Mediterranean ancestries. FMF has been associated with the variations of the MEFV gene, which encodes pyrin protein. Pyrin is expressed in neutrophils, monocytes, dendritic cells, and fibroblasts and is thought to be involved in the regulation of inflammasome function. Most of the disease-associated variations occur in the carboxy-terminal (C-terminal) PRY-SPRY domain of the pyrin protein. The carrier rate is quite high in Eastern Mediterranean populations. Recent observations suggest a more complex pattern of inheritance than a recessive model including some FMF patients with single or no MEFV variations or with dominantly inherited mutations (Masters et al. 2009; Booty et al. 2009; Lachmann 2011; Savic et al. 2012).

The tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) was originally described as familial Hibernian fever. It can be seen in all ethnic groups, but it is more common in Northern European populations. It usually starts during childhood, and inflammatory attacks last a few weeks. During attacks, patients may experience fever, abdominal pain, arthralgia or

Autoinflammatory Diseases, Table 1 Features of common hereditary autoinflammatory diseases (Masters et al. 2009; Lachmann 2011; Savic et al. 2012; Goldbach-Mansky 2012)

	Gene/protein	Ethnicity	Length of attacks	Distinctive clinical features
Autosomal recessively inherited				
FMF	MEFV/Pyrin	Jewish, Armenian, Turkish, Arab	12–72 h	Peritonitis, pleuritis, arthritis, erysipelas-like erythema
HIDS	MVK/ mevalonate kinase	Dutch, Northwestern Europe/All	3–7 days	Abdominal pain, cervical nodes, oral aphthous ulcers, maculopapular rash, arthralgias
DIRA	IL1RN/IL-1Ra	All	Continuous	Pustular skin rash, multifocal osteomyelitis, periostitis
Autosomal dominantly inherited				
TRAPS	TNFRSF1A/ Type 1 TNF receptor	Northern European/All	>5 days, 1–4 weeks	Periorbital edema, headache, abdominal pain, arthralgia/myalgia, fasciitis
Cryopyrin (NLRP3)-associated periodic syndromes				
FCAS	NLRP3/NLRP3	Northern European/All	Usually <24 h	Cold-induced fever, urticarial rash, conjunctivitis, arthralgias
MWS	NLRP3/NLRP3	Northern European/All	24–48 h or continuous	Urticarial rash, conjunctivitis, sensorineural deafness
NOMID	NLRP3/NLRP3	Northern European/All	Continuous with exacerbations	Urticarial rash, bony overgrowth/arthropathy, sensorineural deafness, aseptic meningitis, mental retardation, papilledema
PAPA syndrome	PSTPIP1/ PSTPIP1	Northern European	Days/weeks	Pyoderma gangrenosum, cystic acne, migratory pyogenic arthritis
Blau syndrome	NOD2 (CARD15)	All	Continuous	Granulomatous polyarthritis, uveitis and rash; camptodactyly

FMF familial Mediterranean fever, *HIDS* hyper-IgD and periodic fever syndrome, *DIRA* deficiency of IL-1Ra, *TRAPS* TNF receptor-associated periodic syndrome, *FCAS* familial cold autoinflammatory syndrome, *NOMID* neonatal onset multisystem autoinflammatory disease, *PAPA* pyogenic arthritis, pyoderma gangrenosum and cystic acne syndrome

myalgia with centripetal migration, reticular or serpiginous erythematous rash, periorbital edema, and headache. However, some of the manifestations may be continuous. TRAPS is associated with dominant variations in the type-1 (p55) TNF receptor gene (TNFRSF1A), and most of the disease-associated variants occur in the extracellular part of the receptor, particularly in the first two cysteine-rich domains (McDermott et al. 1999; Lachmann 2011).

Cryopyrin-associated periodic syndromes (CAPS) or cryopyrinopathies consist of three disorders caused by variations in the NLRP3 gene and are associated with overlapping manifestations starting during infancy. A continuum in disease severity may span from the mildest familial cold autoinflammatory syndrome (FCAS) to moderate Muckle-Wells syndrome (MWS) and to the most severe form, neonatal onset multisystem inflammatory disease (NOMID or alternatively named as chronic infantile neurologic cutaneous and articular syndrome, CINCA). FCAS is characterized by cold-induced inflammatory attacks of fever, a neutrophilic urticarial rash, conjunctivitis, and arthralgia, lasting for 24 h or less. MWS has been associated with more severe inflammatory findings lasting 1–2 days which may be complicated by the development of sensorineural deafness and secondary (type AA) amyloidosis. NOMID is associated with continuous inflammation starting at the neonatal period with chronic aseptic meningoencephalitis, developmental retardation, bony overgrowth and deforming arthropathy, deafness, and papilledema/optic

atrophy (Masters et al. 2009; Lachmann 2011). CAPS-related NLRP3 mutations have an autosomal dominant inheritance pattern, but de novo mutations are frequently observed especially in NOMID patients. In almost half of the patients with CAPS phenotype, no NLRP3 mutation can be detected. However, an increasing number of CAPS patients with somatic mosaicism have been observed with parallel deep sequencing (Tanaka et al. 2011).

Mevalonate kinase deficiency (MKD) or hyper-IgD and periodic fever syndrome (HIDS) is a rare autoinflammatory disease with an autosomal recessive inheritance pattern. MKD is more prevalent in the Netherlands, probably due to a founder effect. It usually starts during early childhood with recurrent episodes of fever, cervical lymphadenopathy, abdominal pain, vomiting, and/or diarrhea. Other manifestations may include splenomegaly, arthralgia or arthritis, headache, erythematous macules and papules, as well as oral and genital aphthous ulcers. Elevation of serum IgD levels is not specific for MKD, and it may not be present in all patients. Disease-associated variations are related to mutations in the mevalonate kinase (MVK) gene, which is involved in the biosynthesis of cholesterol, farnesyl, and isoprenoid.

Rarer forms of autoinflammatory syndromes include deficiency of the interleukin-1 receptor antagonist (DIRA), Blau syndrome, pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome, and others (Goldbach-Mansky 2012). DIRA is an autosomal recessive disease and starts at the neonatal period with widespread pustular rash. Inflammatory findings include joint swelling, osteolytic lesions, periostitis, and heterotopic bone formation around the proximal femur. Similarly, a homozygous missense mutation in the IL36RN gene (which encodes the interleukin-36 receptor (IL-36R) antagonist) leads to deficiency of the IL-36R antagonist (DITRA) and is associated with generalized pustular psoriasis. Another pyogenic disease, PAPA syndrome, is associated with mutations in the proline-serine-threonine phosphatase-interacting protein-1 (PSTPIP1) gene and characterized by severe cystic acne, pyoderma gangrenosum, and sterile pyogenic arthritis. Skin and joint findings may develop following minor trauma in patients with PAPA syndrome. Blau syndrome, a NOD2 (nucleotide-binding oligomerization domain 2)-associated autoinflammatory disorder, is characterized by granulomatous uveitis, skin findings, and arthritis. Disease-associated mutations usually occur in the nucleotide-binding domain. Relatively common variations in the leucine-rich repeat domain of the same NOD2 gene have also been associated with Crohn's disease. Additionally, lack of interleukin-10 signaling due to IL10RA or IL10RB mutations has been shown to be causing early-onset inflammatory bowel disorders.

Triggers

In most of the hereditary autoinflammatory disorders, an obvious trigger for exacerbation of inflammation cannot be identified. However, some inflammatory episodes may develop as a result of environmental triggers and gene-environment interactions. Known exogenous triggers include cold exposure in FCAS, vaccinations in HIDS, minor physical trauma in TRAPS, PAPA and DIRA syndromes, strenuous exercise, psychological stress, and menstrual cycles in FMF (Masters et al. 2009; Lachmann 2011; Savic et al. 2012). Also, most of the autoinflammatory disorders show a tendency for ameliorated inflammatory episodes during late adulthood.

Amyloidosis Risk

FMF has been associated with an increased risk for amyloidosis (type AA), and historical data reveals amyloidosis-related nephrotic syndrome and renal failure as the main cause of morbidity and mortality. Development of amyloidosis may be associated with both genetic (MEFV and SAA genotypes) and environmental factors with a higher risk among those with a history of childhood in countries with high infant mortality rates (Touitou et al. 2007). Increased risk of secondary amyloidosis has also been shown in other autoinflammatory disorders, i.e., MWS, NOMID, TRAPS, and even in HIDS and FCAS.

Differential Diagnosis

Exclusion of other possible diagnoses, such as infections, malignancies, and autoimmune disorders, is crucial (Kallinich et al. 2013). Narrowing the differential diagnosis of a patient with autoinflammatory disease is challenging, especially because the clinical features overlap extensively. A few distinctive clinical features, duration of attacks, asymptomatic periods and periodicity of attacks, ancestry, family history, and mode of inheritance may provide some help (Table 1), but it may not be possible to differentiate among these disorders based only on their clinical findings. Genetic diagnosis is also a challenge, and there are several cases with typical manifestations and no variations in the relevant autoinflammatory genes. Also, novel disorders with limited number of cases and newly identified genes continue to be added to the list of autoinflammatory diseases, such as NALP12-associated periodic syndrome (Jéru et al. 2008), and Nakajo-Nishimura syndrome (also known as chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, CANDLE), associated with proteasome protein PSMB8 mutations.

Acquired or Complex Conditions with Autoinflammatory Features

Following the identification of mutations affecting the functions of the inflammasome complexes and other intracellular danger-sensing pathways as the underlying mechanism of hereditary autoinflammatory diseases, similar clinical and inflammatory features have been recognized in the pathogenesis of several acquired conditions. Multifactorial or complex diseases with autoinflammatory features include gout, pseudogout, asbestosis, silicosis, systemic onset juvenile idiopathic arthritis, Behçet's disease, PFAPA (periodic fever with aphthous stomatitis, pharyngitis, and cervical adenopathy) syndrome, Schnitzler syndrome, chronic recurrent multifocal osteomyelitis, SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis) syndrome, type-2 diabetes mellitus, and atherosclerosis. Identification of the relevant inflammatory pathways will not only provide insight into their pathogenesis but may also elucidate new treatment targets (McGonagle et al. 2007; Kastner et al. 2010; Goldbach-Mansky 2012).

Pathogenic Mechanisms

Hereditary autoinflammatory diseases are usually associated with missense sequence variations in a limited number of genes involved in innate immune response. Most mutations are found in the genes encoding proteins involved in intracellular macromolecular complexes (inflammasomes). (An updated list of mutations can be found at the INFEVERS database, http://fmf.igh.cnrs.fr/ISSAID/infevers/). Inflammasomes are formed by NOD-like receptors (NLRs) and retinoic acid-inducible gene-I (RIG-I)-like helicases (RLHs), which function by scanning microbial insults or endogenous danger signals similarly to Toll-like receptors (TLRs). Activation of TLRs causes production of pro-IL-1β and pro-IL-18 through NF-κB activation. However, a second signal, inducing the assembly of cytosolic inflammasome platforms, is necessary for the autocatalytic regulation of inflammatory caspases, mainly caspase-1, and proteolytic activation of its main substrates, IL-1β, IL-18, and possibly IL-33 (Martinon et al. 2009; Hoffman and Brydges 2011; Lamkanfi and Dixit 2012; Strowig et al. 2012).

The recognition mechanisms of intracellular threats by inflammasome proteins, through conserved pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), have not yet been clarified. A decrease in intracellular potassium levels, activation of purinoreceptor P2X7 by extracellular ATP, or pore-forming bacterial toxins have been shown to induce inflammasome assembly (Martinon et al. 2009; Lamkanfi and Dixit 2012; Strowig et al. 2012).

Mutations in the NLRP3 gene are associated with CAPS, and these dominantly inherited gain-of-function variations result in increased IL-1β production by mononuclear cells in response to microbial or environmental (such as cold temperature) triggers, even in the absence of a second

signal like extracellular ATP or potassium efflux. Constitutively hyperactive inflammasome and spontaneous IL-1β secretion have been involved in the pathogenesis of CAPS manifestations, and blocking of IL-1β activity has provided dramatic improvement.

Deregulated inflammasome activation has also been suggested in other hereditary autoinflammatory conditions, such as FMF, or acquired conditions like gout, pseudogout, or other crystal-induced diseases including silicosis and asbestosis. Pyrin has been considered a regulatory inflammasome protein, but it has not yet been clarified whether FMF-associated variations, mainly occurring in the C-terminal PRY-SPRY domain, are loss-of-function or gain-of-function mutations (Chae et al. 2011; Hesker et al. 2012). Pyrin interacts with ASC, an adaptor protein, and with caspase-1. Additionally, mutations in the PSTPIP1 gene, which encodes another pyrin-interacting protein, are associated with PAPA syndrome. PAPA-related mutations result in increased pyrin affinity, but how it affects inflammasome function is not yet known.

Different mechanisms have been proposed to explain the autoinflammatory features of TRAPS. Of note, TRAPS is associated with mutations of TNFRSF1A, a gene that does not produce an inflammasome-related protein. Dominant mutations in TNFRSF1A have been suggested to be associated with prolonged signaling due to abnormal type 1 TNF receptor trafficking and its intracellular retention rather than the original hypothesis of defective receptor shedding. Also, hyperresponsiveness to LPS stimulation of TRAPS patients has been shown to be associated with higher activation of mitogen-activated protein kinase (MAPK) due to mitochondria-derived reactive oxygen species (ROS). ROS may also play a critical role in NLRP3-associated disorders, as a final common pathway of different secondary signals (Simon et al 2010; Bulua et al. 2011).

The pathogenesis of HIDS, which is associated with mutations in the MVK gene, is even less clear. Recessively inherited MVK variations result in a defect in the metabolic mevalonate pathway, which leads to a deficiency of geranylgeranyl pyrophosphate and aberrant activation of Rac1. This GTPase defect has been suggested to cause inflammasome activation (van der Burgh et al. 2012).

In addition to the mechanisms associated with constitutive or induced hyperactivation of inflammasomes, other pathogenic mechanisms of autoinflammatory disease have been proposed. These include NF-κB activation (Crohn's disease), protein misfolding, activation of complement component H (atypical hemolytic-uremic syndrome, age-related macular degeneration), abnormal cytokine signaling (Cherubism), macrophage activation (familial hemophagocytic lymphohistiocytosis), and proteasome disability (Nakajo-Nishimura syndrome) (Masters et al. 2009; Kastner et al. 2010).

The revised definition of autoinflammatory diseases has fuelled studies on innate immune recognition and regulation of the inflammatory response. On the other hand, both innate and adaptive immune responses are involved at different degrees in the pathogenesis of many complex inflammatory diseases, suggesting a continuum between the autoinflammatory and the autoimmune disorders (McGonagle et al. 2007).

Treatment

The management of autoinflammatory disorders is directed both at controlling the acute attacks and preventing recurrences and complications. For acute attacks, treatment is usually symptomatic and may include antipyretics and nonsteroidal anti-inflammatory drugs. Attacks of TRAPS patients may respond to high-dose corticosteroids.

Colchicine has long been known as a potent anti-inflammatory drug, and it has been used as an effective and safe medication in FMF patients for the prevention of exacerbations and secondary amyloidosis. However, some FMF patients do not tolerate colchicine treatment or tolerated doses may be inadequate to control disease activity.

In CAPS patients, blockade of IL-1 activity by anakinra (recombinant IL-1 receptor antagonist), rilonacept (IL-1 receptor fusion protein), or anti-IL-1β monoclonal antibody canakinumab, has been shown to be an effective and safe treatment (Goldbach-Mansky 2012).

No treatment has been licensed for TRAPS and HIDS patients, but IL-1 blockade may be a helpful option. Efficacy of IL-1 blockade has also been shown in FMF patients with inadequate response to colchicine and in some complex autoinflammatory conditions, such as systemic juvenile idiopathic arthritis and gout (Goldbach-Mansky 2012).

Although TRAPS is associated with defective TNF receptor signaling, the role of anti-TNF drugs is controversial in its treatment. Some TRAPS patients may respond to etanercept, but worsening of symptoms was observed with infliximab.

Biologic agents that block IL-6 signaling may also be an option for the treatment of resistant patients with hereditary or acquired autoinflammatory diseases.

Conclusions

Hereditary autoinflammatory diseases have been characterized by recurrent, self-limited inflammatory episodes predominantly mediated by genetic variations affecting the innate immune system. However, similar inflammatory pathways are recognized to be associated with the pathogenesis of a growing number of complex disorders. Autoinflammatory diseases are usually linked to spontaneous or induced hyperactivity of cytosolic multiprotein inflammasome complexes, but other genetic variations affecting proteins that are involved in intracellular recognition and processing of danger signals may also play a role in the increased inflammatory response. Identification of IL-1 as the critical inflammatory mediator has led to the development of effective treatment options for several hereditary autoinflammatory disorders, and it also has provided new therapeutic opportunities for complex conditions with autoinflammatory features.

Cross-References

- ▶ Environment and Autoimmunity
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Booty MG, Chae JJ, Masters SL, Remmers EF, Barham B, Le JM, Barron KS, Holland SM, Kastner DL, Aksentijevich I. Familial Mediterranean fever with a single MEFV mutation: where is the second hit? Arthritis Rheum. 2009;60:1851–61.

Bulua AC, Simon A, Maddipati R, Pelletier M, Park H, Kim KY, Sack MN, Kastner DL, Siegel RM. Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). J Exp Med. 2011;208:519–33.

Chae JJ, Cho YH, Lee GS, Cheng J, Liu PP, Feigenbaum L, Katz SI, Kastner DL. Gain-of-function Pyrin mutations induce NLRP3 protein-independent interleukin-1β activation and severe autoinflammation in mice. Immunity. 2011;34:755–68.

Goldbach-Mansky R. Immunology in clinic review series; focus on autoinflammatory diseases: update on monogenic autoinflammatory diseases: the role of interleukin (IL)-1 and an emerging role for cytokines beyond IL-1. Clin Exp Immunol. 2012;167:391–404.

Hesker PR, Nguyen M, Kovarova M, Ting JP, Koller BH. Genetic loss of murine pyrin, the familial Mediterranean fever protein, increases interleukin-1β levels. PLoS One. 2012;7:e51105.

Hoffman HM, Brydges SD. Genetic and molecular basis of inflammasome-mediated disease. J Biol Chem. 2011;286:10889–96.

Jéru I, Duquesnoy P, Fernandes-Alnemri T, Cochet E, Yu JW, Lackmy-Port-Lis M, Grimprel E, Landman-Parker J, Hentgen V, Marlin S, McElreavey K, Sarkisian T, Grateau G, Alnemri ES, Amselem S. Mutations in NALP12 cause hereditary periodic fever syndromes. Proc Natl Acad Sci USA. 2008;105:1614–9.

Kallinich T, Gattorno M, Grattan CE, de Koning HD, Traidl-Hoffmann C, Feist E, Krause K, Lipsker D, Navarini AA, Maurer M, Lachmann HJ, Simon A. Unexplained recurrent fever: when is autoinflammation the explanation? Allergy. 2013. doi:10.1111/all.12084.

Kastner DL, Aksentijevich I, Goldbach-Mansky R. Autoinflammatory disease reloaded: a clinical perspective. Cell. 2010;140:784–90.

Lachmann HJ. An approach to the patient with a periodic fever syndrome. Clin Exp Immunol. 2011;165:301–9.

Lamkanfi M, Dixit VM. Inflammasomes and their roles in health and disease. Annu Rev Cell Dev Biol. 2012;28:137–61.

Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. Annu Rev Immunol. 2009;27:229–65.

Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. Annu Rev Immunol. 2009;27:621–68.

McDermott MF, Aksentijevich I, Galon J, McDermott EM, Ogunkolade BW, Centola M, et al. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. Cell. 1999;97:133–44.

McGonagle D, Savic S, McDermott MF. The NLR network and the immunological disease continuum of adaptive and innate immune-mediated inflammation against self. Semin Immunopathol. 2007;29:303–13.

Savic S, Dickie LJ, Battellino M, McDermott MF. Familial Mediterranean fever and related periodic fever syndromes/autoinflammatory diseases. Curr Opin Rheumatol. 2012;24:103–12.

Simon A, Park H, Maddipati R, Lobito AA, Bulua AC, Jackson AJ, Chae JJ, Ettinger R, de Koning HD, Cruz AC, Kastner DL, Komarow H, Siegel RM. Concerted action of wild-type and mutant TNF receptors enhances inflammation in TNF receptor 1-associated periodic fever syndrome. Proc Natl Acad Sci USA. 2010;107:9801–6.

Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature. 2012;481:278–86.

Tanaka N, Izawa K, Saito MK, Sakuma M, Oshima K, Ohara O, Nishikomori R, Morimoto T, Kambe N, Goldbach-Mansky R, Aksentijevich I, de Saint BG, Neven B, van Gijn M, Frenkel J, Aróstegui JI, Yagüe J, Merino R, Ibañez M, Pontillo A, Takada H, Imagawa T, Kawai T, Yasumi T, Nakahata T, Heike T. High incidence of NLRP3 somatic mosaicism in patients with chronic infantile neurologic, cutaneous, articular syndrome: results of an International Multicenter Collaborative Study. Arthritis Rheum. 2011;63:3625–32.

Touitou I, Sarkisian T, Medlej-Hashim M, Tunca M, Livneh A, Cattan D, Yalçinkaya F, Ozen S, Majeed H, Ozdogan H, Kastner D, Booth D, Ben-Chetrit E, Pugnère D, Michelon C, Séguret F, Gershoni-Baruch R, International Study Group for Phenotype-Genotype Correlation in Familial Mediterranean Fever. Country as the primary risk factor for renal amyloidosis in familial Mediterranean fever. Arthritis Rheum. 2007;56:1706–12.

van der Burgh R, Ter Haar NM, Boes ML, Frenkel J. Mevalonate kinase deficiency, a metabolic autoinflammatory disease. Clin Immunol. 2012; S1521–6616(12):00236-7.

B

B Cell Central Tolerance: Controlling Self-Reactive B Cells in the Bone Marrow

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Synonyms

B cell negative selection; B cell tolerance; Self-tolerance; Tolerance mechanisms

Definition

B cell central tolerance is defined as a process by which negative selection mechanisms operate during early stages of B cell development in the bone marrow (BM) to diminish the incidence of autoimmunity by preventing self-reactive immature B cells to become mature effector B cells.

Introduction

The ability of B cells to produce antibodies against almost any foreign antigen is the outcome of random assembly of immunoglobulin (Ig) genes. The resultant huge diversity of the B cell repertoire enables our body to thwart pathogens. However, the randomness of Ig gene rearrangement process leads in high frequency to the generation of self-reactive B cells that might mature and enter the secondary lymphoid organs and tissues outside the BM. Without efficient selection processes to impose tolerance of the immune system to self-antigens, this could result in an autoimmune response and production of autoantibodies, some of which may be pathogenic. Thus, autoimmunity, defined as various physiological disorders characterized in unwanted response against self-tissues, reflects a breakdown in self-tolerance.

Self-Reactive B Cells and Autoimmunity

B lymphocytes are important mediators of autoimmunity as autoantibodies are primary cause of many systemic (such as systemic lupus erythematosus – SLE and rheumatoid arthritis – RA) and organ-specific (such as Graves' disease) diseases (Azulay-Debby and Melamed 2007). B cells may also promote autoimmunity as they function to regulate differentiation of effector T cells (Mauri and Bosma 2012; Mizoguchi and Bhan 2006), secrete cytokines which affect innate and adaptive immune cells (Kwan-Morley and Albert 2007; Lipsky 2001; Lund and Randall 2010), and present antigens (Chan et al. 1999; Salinas et al. 2013). Autoreactive

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B cell clones are parts of the normal peripheral repertoire (Koenig-Marrony et al. 2001; Rice et al. 2005), but they are kept in low frequency and mostly in an unresponsive state due to central and peripheral mechanisms of self-tolerance (Edry and Melamed 2004; Klinman 1996; Nossal 1994; Rajewsky 1996; Shlomchik et al. 2001). The exact cause for breakdown of these tolerance mechanisms is not fully understood. Nevertheless, antigenic mimicry and bystander activation of autoreactive clones have been proposed (Christen and von Herrath 2004; Marrack et al. 2001; Shlomchik et al. 2001). Prolongation of immature B cell short half-life also favors B cell–driven autoimmunity (von Boehmer and Melchers 2010). Immune dysregulation, leading to tolerance breakdown, is a precondition; yet, genetic predisposition and environmental factors are also major contributors to the development of autoimmune disorders (Ermann and Fathman 2001).

B Cell Development

B lymphocytes are blood cells that develop in the BM (a central lymphoid organ) and migrate to the spleen (periphery) to complete their maturation (Osmond 1991; Rajewsky 1996). Each B cell expresses a unique surface receptor for antigen (B cell receptor; BCR). During development in the BM, B cells proliferate and progress through a highly regulated process that is guided by successive attempts to assemble and express immunoglobulin heavy (IgH) and light (IgL) chain genes to form the BCR. Several selection checkpoints have been discovered, which allow (positive selection) or prevent (negative selection) this developmental progression (Hartley et al. 1993; Rolink et al. 2001). Upon completion of BCR gene rearrangement and expression at the progenitor (pro) and precursor (pre) stages, B cells differentiate into the immature stage where the newly generated BCR is expressed as surface IgM (sIgM) (Martin and Kearney 2000). The BCR of immature B cells is critical not only for negative selection but also for generation of appropriate tonic signals to promote B cell maturation and migration from the BM to the spleen, a process referred as positive selection (Bannish et al. 2001; Keren et al. 2004; Kraus et al. 2001; Levine et al. 2000). Immature B cells that emigrate from the BM to the periphery are called transitional B cells, and are subjected to further selection steps (Meffre et al. 2000). Only 3 % of the immature B cells generated in the BM become mature B cells (Osmond 1991; Rajewsky 1996). This stringent selection is attributed in part to the fact that the majority (55–75 %) of the BCRs, randomly assembled in the BM, are self-reactive (Nemazee 1996; Wardemann et al. 2003). Most of this self-reactivity is extinguished by central tolerance mechanisms (Azulay-Debby and Melamed 2007).

Central Tolerance Mechanisms

The expression of sIgM by immature B cells is a critical selection check-point in B cell developmental progression. If the strength of BCR cross-linking and intracellular signaling exceeds a certain threshold by binding to a self-antigen, the immature B cell rapidly internalizes the aberrant BCR and temporarily aborts its maturation program (Goodnow et al. 2005). This B cell autoreactivity is extinguished in the BM mainly by three cellular mechanisms.

First, cells displaying self-reactive receptors can be triggered to die, as originally envisaged in Burnet's concept of *clonal deletion* (Goodnow et al. 2005), in a process called activation-induced cell death (AICD) (Donjerkovic and Scott 2000). It has been shown that clonal deletion is a major mechanism for membrane-immobilized autoantigens (Klinman 1996; Nemazee and Burki 1989; Norvell et al. 1995).

Second, autoreactive B cells can escape clonal deletion by revising their antigen receptors through a process of secondary Ig gene rearrangements at the IgL locus, a mechanism known as *receptor editing* (Nemazee 2000; Nemazee and Weigert 2000).

Third, intrinsic biochemical and gene-expression changes can reduce the ability of the cells to be triggered by self-reactive receptors. This mechanism is termed *clonal anergy*

(Goodnow et al. 2005), and is generally imposed by soluble low-avidity self-antigens (Goodnow et al. 1988; Nossal 1996).

Clonal Deletion

Immature B cells mount heighten sensitivity to AICD imposed by BCR signaling relative to mature B cells (Meffre et al. 2000). This has been demonstrated following addition of anti-BCR antibodies to BM cultures containing both immature and mature recirculating B cells. In these experiments, a high rate of immature B cell apoptosis was observed whereas mature B cells were spared (King and Monroe 2000). In vivo experiments revealed that elimination of immature B cells occurs within 1–2 days, either in the BM or shortly after arriving in the spleen (Hartley et al. 1993; Niiro and Clark 2002). A major cause for the heighten sensitivity to AICD is the increased expression of *Pten* in immature B cells relative to the levels found in mature B cells (Cheng et al. 2009). *Pten* encodes for a lipid phosphatase that directly antagonizes the activity of pro-survival phosphoinositide 3-kinase (PI3K) by dephosphorylating PIP_3 into PIP_2 (Suzuki et al. 2003). The pro-apoptotic Fas/FasL pathway does not appear to play a role in the BCR-induced death of immature B cells (King and Monroe 2000). However, clonal deletion by AICD involves an increased expression of the pro-apoptotic protein BIM (BCL-2- interacting mediator of cell death) (Strasser and Bouillet 2003) as well as mitochondria dysfunction, which facilitates release of cytochrome *c* and activation of caspases (Niiro and Clark 2002). In addition, autoreactive immature B cells maintain low levels of receptors for B cell activating factor (BAFF), a circulating cytokine required for survival of peripheral B cells (Mackay et al. 2003).

Receptor Editing

B cells expressing defective and/or self-reactive receptors may undergo secondary Ig gene rearrangement at the IgL locus to alter the specificity of their receptor, rather than undergoing a default apoptosis (Azulay-Debby and Melamed 2007; Edry and Melamed 2004). This salvage mechanism was first characterized in central tolerance of immature B cells undergoing negative selection, and termed receptor editing (Gay et al. 1993; Tiegs et al. 1993). Receptor editing is facilitated by replacing the entire preexisting VκJκ through secondary recombination of an upstream Vκ to a downstream Jκ (Nemazee 2000). Alternatively, recombination of the conserved element known as recombining sequence (RS) into the Cκ inactivates the locus and allows IgL rearrangement and expression from the other kappa allele or from the lambda locus from either allele (Retter and Nemazee 1998). Receptor editing has been demonstrated by re-induction or continuous expression of RAG-1 and RAG-2, (recombination-activating gene 1 and 2, which encode the core enzymes for V(D)J recombination), resulting in alteration in Jκ gene usage and increased λ -expressing cells in normal and transgenic (Tg) immature B cells undergoing negative selection (Melamed and Nemazee 1997; Radic et al. 1993). Central tolerance mouse models indicate that autoreactive B cells are very efficiently rescued by receptor editing at the IgL locus, although multiple rearrangements are often required until the appropriate Vκ is selected (Li et al. 2001). Studies have shown that receptor editing is regulated developmentally as immature B cells advancing in their development lose the ability to undergo receptor editing and concomitantly acquire sensitivity to antigen-mediated apoptosis (Melamed et al. 1998). Thus, negative selection of immature B cells in the BM is mainly facilitated by receptor editing whereas that of transitional B cells in the spleen is facilitated by AICD (Sandel and Monroe 1999). Recently, it has been demonstrated that receptor editing appears to have an important contribution to positive selection of B cells as well, since BCR that fails to gain appropriate tonic signals undergoes extensive receptor editing in the BM (Diamant et al. 2005; Shivtiel et al. 2002; Tze et al. 2005).

Secondary rearrangements have also been shown to occur in high frequency at the IgH locus. These rearrangements utilize a conserved heptameric sequence that is embedded in the VH to allow a recombination of an upstream VH gene into the existing VDJ (Nemazee 2000). It is

thought that receptor editing at the IgH locus is activated in pro-B cells where it remediates impaired assembly and/or signaling of the pre-BCR for positive selection, rather than it avoids negative selection induced by autoreactivity (Azulay-Debby and Melamed 2007).

Clonal Anergy

Self-reactive B cells that fail to edit their receptors are deleted or anergized. Anergy was originally observed in cultures of developing B cells exposed to intermediate concentrations of anti-IgM antibodies. This allowed B cell development but abrogated normal B cell function, as determined by decreased proliferation and antibody production upon mitogen exposure (Meffre et al. 2000). Using Ig transgenic mouse models, it has been demonstrated that chronic exposure of immature B cells to low affinity antigens in the BM can lead to clonal anergy characterized by 20–50-fold reduction in membrane IgM expression levels, and by incompetent signaling through the BCR (Fulcher and Basten 1994). Loss-of-function mutations in a series of molecules in the BCR signaling pathway have also indicated that BCR signaling is downregulated in anergic B cells. The resultant "paralyzed" anergic B cells are short-lived, and fail to develop from the immature to the long-lived B cell compartment in the spleen (Meffre et al. 2000). Activation of anergic B cells through their BCR stimulates AICD rather than proliferation (Goodnow 1996). The intrinsic biochemical changes that govern cellular mechanisms of anergy in the cells displaying self-reactive receptors are reversible if the BCR stops binding to the self-antigen, as would occur if the BCR is edited to lose self-reactivity through somatic hypermutation in germinal centers (Goodnow et al. 2005).

Summary

Self-tolerance in the B lineage is regulated during the development in the BM and the spleen in order to prevent autoimmunity and to maintain the integrity of the organism (Diamant and Melamed 2004). Clonal deletion, receptor editing, and clonal anergy are the main mechanisms known to impose B cell central tolerance in the BM. Yet, these mechanisms are incomplete, and probably affect only the highest affinity self-reactive lymphocytes (Shlomchik et al. 2001). Likewise, a certain proportion of autoreactive B cells may bind self-antigen with too low affinity to trigger an autoimmune response, but may bind strongly enough to invading pathogens to exert a protective host-defense effect (von Boehmer and Melchers 2010). Experimental and human data clearly indicate that leaky central tolerance increases the risk for subsequent development of autoimmune disease but that a number of additional factors control this progression (Salinas et al. 2013). Autoreactive B cells that escape central tolerance mechanisms are actively regulated and prevented from effector functions in the periphery (Shlomchik et al. 2001), and failure to maintain this regulation may progress into autoimmunity.

Cross-References

- ▶ B Cell Tolerance
- ▶ B Cell Trafficking
- ▶ BCR Signaling
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Azulay-Debby H, Melamed D. B cell receptor editing in tolerance and autoimmunity. Front Biosci. 2007;12:2136–47.

Bannish G, Fuentes-Panana EM, Cambier JC, Pear WS, Monroe JG. Ligand-independent signaling functions for the B lymphocyte antigen receptor and their role in positive selection during B lymphopoiesis. J Exp Med. 2001;194:1583–96.

Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. J Exp Med. 1999;189:1639–48.

Cheng S, Hsia CY, Feng B, Liou ML, Fang X, Pandolfi PP, et al. BCR-mediated apoptosis associated with negative selection of immature B cells is selectively dependent on Pten. Cell Res. 2009;19:196–207.

Christen U, von Herrath MG. Initiation of autoimmunity. Curr Opin Immunol. 2004;16:759–67.
Diamant E, Melamed D. Class switch recombination in B lymphopoiesis: a potential pathway for B cell autoimmunity. Autoimmun Rev. 2004;3:464–9.
Diamant E, Keren Z, Melamed D. CD19 regulates positive selection and maturation in B lymphopoiesis: lack of CD19 imposes developmental arrest of immature B cells and consequential stimulation of receptor editing. Blood. 2005;105:3247–54.
Donjerkovic D, Scott DW. Activation-induced cell death in B lymphocytes. Cell Res. 2000;10:179–92.
Edry E, Melamed D. Receptor editing in positive and negative selection of B lymphopoiesis. J Immunol. 2004;173:4265–71.
Ermann J, Fathman CG. Autoimmune diseases: genes, bugs and failed regulation. Nat Immunol. 2001;2:759–61.
Fulcher DA, Basten A. Reduced life span of anergic self-reactive B cells in a double-transgenic model. J Exp Med. 1994;179:125–34.
Gay D, Saunders T, Camper S, Weigert M. Receptor editing: an approach by autoreactive B cells to escape tolerance. J Exp Med. 1993;177:999–1008.
Goodnow CC. Balancing immunity and tolerance: deleting and tuning lymphocyte repertoires. Proc Natl Acad Sci USA. 1996;93:2264–71.
Goodnow CC, Crosbie J, Adelstein S, Lavoie TB, Smith-Gill SJ, Brink RA, et al. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature. 1988;334:676–82.
Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. Nature. 2005;435:590–7.
Hartley SB, Cooke MP, Fulcher DA, Harris AW, Cory S, Basten A, et al. Elimination of self-reactive B lymphocytes proceeds in two stages: arrested development and cell death. Cell. 1993;72:325–35.
Keren Z, Diamant E, Ostrovsky O, Bengal E, Melamed D. Modification of ligand-independent B cell receptor tonic signals activates receptor editing in immature B lymphocytes. J Biol Chem. 2004;279:13418–24.
King LB, Monroe JG. Immunobiology of the immature B cell: plasticity in the B-cell antigen receptor-induced response fine tunes negative selection. Immunol Rev. 2000;176:86–104.
Klinman NR. The "clonal selection hypothesis" and current concepts of B cell tolerance. Immunity. 1996;5:189–95.
Koenig-Marrony S, Soulas P, Julien S, Knapp AM, Garaud JC, Martin T, et al. Natural autoreactive B cells in transgenic mice reproduce an apparent paradox to the clonal tolerance theory. J Immunol. 2001;166:1463–70.
Kraus M, Pao LI, Reichlin A, Hu Y, Canono B, Cambier JC, et al. Interference with immunoglobulin (Ig)alpha immunoreceptor tyrosine-based activation motif (ITAM) phosphorylation modulates or blocks B cell development, depending on the availability of an Igbeta cytoplasmic tail. J Exp Med. 2001;194:455–69.
Kwan-Morley J, Albert D. B-cell inhibitors as therapy for rheumatoid arthritis: an update. Curr Rheumatol Rep. 2007;9:401–6.
Levine MH, Haberman AM, Sant'Angelo DB, Hannum LG, Cancro MP, Janeway Jr CA, et al. A B-cell receptor-specific selection step governs immature to mature B cell differentiation. Proc Natl Acad Sci USA. 2000;97:2743–8.
Li H, Jiang Y, Prak EL, Radic M, Weigert M. Editors and editing of anti-DNA receptors. Immunity. 2001;15:947–57.
Lipsky PE. Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. Nat Immunol. 2001;2:764–6.
Lund FE, Randall TD. Effector and regulatory B cells: modulators of CD4(+) T cell immunity. Nat Rev Immunol. 2010;10:236–47.
Mackay F, Schneider P, Rennert P, Browning J. BAFF AND APRIL: a tutorial on B cell survival. Annu Rev Immunol. 2003;21:231–64.
Marrack P, Kappler J, Kotzin BL. Autoimmune disease: why and where it occurs. Nat Med. 2001;7:899–905.
Martin F, Kearney JF. Positive selection from newly formed to marginal zone B cells depends on the rate of clonal production, CD19, and btk. Immunity. 2000;12:39–49.
Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol. 2012;30:221–41.
Meffre E, Casellas R, Nussenzweig MC. Antibody regulation of B cell development. Nat Immunol. 2000;1:379–85.
Melamed D, Nemazee D. Self-antigen does not accelerate immature B cell apoptosis, but stimulates receptor editing as a consequence of developmental arrest. Proc Natl Acad Sci USA. 1997;94:9267–72.
Melamed D, Benschop RJ, Cambier JC, Nemazee D. Developmental regulation of B lymphocyte immune tolerance compartmentalizes clonal selection from receptor selection. Cell. 1998;92:173–82.
Mizoguchi A, Bhan AK. A case for regulatory B cells. J Immunol. 2006;176:705–10.
Nemazee D. Antigen receptor 'capacity' and the sensitivity of self-tolerance. Immunol Today. 1996;17:25–9.
Nemazee D. Receptor selection in B and T lymphocytes. Annu Rev Immunol. 2000;18:19–51.
Nemazee DA, Burki K. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. Nature. 1989;337:562–6.
Nemazee D, Weigert M. Revising B cell receptors. J Exp Med. 2000;191:1813–7.
Niiro H, Clark EA. Regulation of B-cell fate by antigen-receptor signals. Nat Rev Immunol. 2002;2:945–56.
Norvell A, Mandik L, Monroe JG. Engagement of the antigen-receptor on immature murine B lymphocytes results in death by apoptosis. J Immunol. 1995;154:4404–13.

Nossal GJ. Negative selection of lymphocytes. Cell. 1994;76:229–39.
Nossal GJ. Clonal anergy of B cells: a flexible, reversible, and quantitative concept. J Exp Med. 1996; 183:1953–6.
Osmond DG. Proliferation kinetics and the lifespan of B cells in central and peripheral lymphoid organs. Curr Opin Immunol. 1991;3:179–85.
Radic MZ, Erikson J, Litwin S, Weigert M. B lymphocytes may escape tolerance by revising their antigen receptors. J Exp Med. 1993; 177:1165–73.
Rajewsky K. Clonal selection and learning in the antibody system. Nature. 1996;381:751–8.
Retter MW, Nemazee D. Receptor editing occurs frequently during normal B cell development. J Exp Med. 1998;188:1231–8.
Rice JS, Newman J, Wang C, Michael DJ, Diamond B. Receptor editing in peripheral B cell tolerance. Proc Natl Acad Sci USA. 2005;102:1608–13.
Rolink AG, Schaniel C, Andersson J, Melchers F. Selection events operating at various stages in B cell development. Curr Opin Immunol. 2001;13:202–7.
Salinas GF, Braza F, Brouard S, Tak PP, Baeten D. The role of B lymphocytes in the progression from autoimmunity to autoimmune disease. Clin Immunol. 2013;146:34–45.
Sandel PC, Monroe JG. Negative selection of immature B cells by receptor editing or deletion is determined by site of antigen encounter. Immunity. 1999;10:289–99.
Shivtiel S, Leider N, Sadeh O, Kraiem Z, Melamed D. Impaired light chain allelic exclusion and lack of positive selection in immature B cells expressing incompetent receptor deficient of CD19. J Immunol. 2002;168:5596–604.
Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol. 2001;1:147–53.
Strasser A, Bouillet P. The control of apoptosis in lymphocyte selection. Immunol Rev. 2003;193:82–92.
Suzuki A, Kaisho T, Ohishi M, Tsukio-Yamaguchi M, Tsubata T, Koni PA, et al. Critical roles of Pten in B cell homeostasis and immunoglobulin class switch recombination. J Exp Med. 2003;197:657–67.
Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone marrow B cells. J Exp Med. 1993;177:1009–20.
Tze LE, Schram BR, Lam KP, Hogquist KA, Hippen KL, Liu J, et al. Basal immunoglobulin signaling actively maintains developmental stage in immature B cells. PLoS Biol. 2005;3:e82.
von Boehmer H, Melchers F. Checkpoints in lymphocyte development and autoimmune disease. Nat Immunol. 2010;11:14–20.
Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science. 2003;301:1374–7.

B Cell Tolerance

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Synonyms

B cell clonal deletion; B cell peripheral tolerance; Receptor editing

Definition

B cell tolerance refers to mechanism by which B cells are prevented from inducing immune responses to self-antigens.

Introduction

Tolerance at the B cell level is at least as important as T cell tolerance for a number of reasons. At least 75 % of the starting human B cell repertoire in the bone marrow is self-reactive (Wardemann et al. 2003). Multivalent membrane-bound antigens can activate self-reactive B cells in a T-independent manner, and therefore, B cells that recognize such antigens need to be tolerized independently of T cell tolerance mechanisms (see entries ▶ B Cell Central Tolerance: Controlling Self-reactive B Cells in the Bone Marrow and ▶ B Cell Tolerance). Even weakly self-reactive B cells can be induced to undergo somatic hypermutation if they receive T cell help so such B cells must be tolerized. Helper T cells that are specific for foreign antigens can be easily subverted to help B cells that recognize self-antigens, if a foreign antigen forms a complex with a self-antigen. Under these circumstances, a self-reactive B cell may internalize the self-antigen complexed to a foreign protein and present foreign peptides to a T cell

clone and thus be driven into a T-dependent response that can drive autoimmunity (see entry ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis).

Tolerance mechanisms in the B lineage can be divided into central and peripheral categories. Central B cell tolerance refers to tolerance that occurs in developing or immature B lymphocytes in central or generative organs – primarily the bone marrow. Peripheral tolerance occurs in mature B lymphocytes in peripheral lymphoid organs.

Central B Cell Tolerance

The major mechanism of central B cell tolerance is receptor editing (Tiegs et al. 1993; Gay et al. 1993; Pelanda et al. 1997; Hippen et al. 2005). Other mechanisms include clonal deletion (Nemazee and Burki 1989; Hartley et al. 1991) and anergy (Goodnow et al. 1988). (See entry ▶ B Cell Central Tolerance: Controlling Self-reactive B Cells in the Bone Marrow.)

Receptor Editing

Strongly autoreactive immature B cells that encounter self-antigen in the bone marrow can be induced to revert to the small pre-B stage of development wherein Rag-1 and Rag-2 (recombinase-activating genes 1 and 2) are reexpressed and initially the Igκ light chain locus is made accessible for recombination. The re-induction of V(D) J recombination results in additional rearrangements at the κ locus that result in the deletion of the original self-reactive κ light chain. The number of "chances" for a productive V-J recombination event to occur at the κ light chain locus varies depending on which Jκ gene is initially involved in the rearrangement process and whether a nonproductive rearrangement already exists on one of the two chromosomes. If the self-reactive B cell fails to make a new productive κ light chain rearrangement, signals from the BCR induce nuclear proteins to delete the entire κ light chain locus on both chromosomes, resulting in rearrangements being attempted at the λ light chain locus. This process of deleting a self-reactive rearranged κ light chain gene and inducing a new productive κ or λ light chain rearrangement is known as receptor editing.

Clonal Deletion

Autoreactive B cells can be clonally deleted by apoptosis. This typically occurs at the transitional B cells stage in the spleen just before immature B cells mature into follicular or marginal zone B cells. Evidence from B cell receptor transgenic animals suggests that clonal deletion is a back-up mechanism that facilitates the induction of central tolerance whenever receptor editing proves insufficient.

Clonal Anergy

Clonal anergy in B cells refers to a state of unresponsiveness generally induced by the chronic exposure of immature and mature B cells to an antigen, usually a soluble protein antigen. Repeated B cell receptor triggering leads to a state of enhanced dependency on BAFF/Blys for survival. Anergic B cells do not compete as well as naive B cells for BAF in follicles and therefore have shortened life spans.

Peripheral B Cell Tolerance

Peripheral B cell tolerance is mediated by a number of mechanisms. These include clonal deletion and anergy as discussed above in the context of central tolerance but also include the phenomenon of clonal ignorance and potentially tolerance mediated by regulatory T cells (Tregs).

Clonal Ignorance Mediated by Siglecs

One of the pathways of peripheral tolerance is mediated by inhibitory receptors of the Siglec family (Cornall et al. 1998; Pao et al. 2007; O'Keefe et al. 1999; Pillai et al. 2009). Siglecs are sialic acid binding lectins that contain Ig domains. The best studied Siglec in the

B lineage is CD22. CD22 contains extracellular Ig domains, the two outermost of which bind to α2-6-linked sialic acid on *N*-glycans. This Siglec contains cytosolic ITIMs (immunoreceptor tyrosine-based inhibitory motifs) that are exposed upon ligation and phosphorylated by Lyn. The phosphorylated ITIM tyrosines recruit the SH2 (Src homology 2) domain-containing tyrosine phosphatase SHP-1 (SH2 domain-containing phosphatase-1) which dampens B cell receptor signaling. An autoimmune phenotype is observed in mice harboring mutations in Lyn. A similar phenotype is observed when SHP-1 is conditionally mutated in the B lineage. This pathway mediates B cell tolerance in the periphery.

Tolerance in Germinal Centers

In patients with the autoimmune lymphoproliferative syndrome, mutations in Fas lead to a break in B cell tolerance. Germinal center B cells express high levels of Fas. It is presumed that B cells that are of lower affinity against foreign antigens as well as some self-reactive B cells are eliminated in germinal centers in a Fas-dependent manner. It is unclear if T follicular helper cells express sufficient levels of Fas ligand to mediate this elimination event.

Other Mechanism of Peripheral B Cell Tolerance

There is some evidence supporting peripheral B cell deletion by self-antigens, as well as evidence for anergy (discussed above). Regulatory T cells may also exert peripheral B cell tolerance in a poorly understood way.

Cross-References

- ▶ BCR Signaling
- ▶ Environment and Autoimmunity
- ▶ Epigenetics in Autoimmunity
- ▶ Micro-RNA in Autoimmunity
- ▶ Regulatory B Cells
- ▶ Repertoire Selection
- ▶ Rheumatoid Arthritis, Genetics
- ▶ Systemic Lupus Erythematosus, Genetics
- ▶ Systemic Lupus Erythematosus, Pathogenesis

References

Cornall RJ, Cyster JG, Hibbs ML, Dunn AR, Otipoby KL, Clark EA, Goodnow CC. Polygenic autoimmune traits: Lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. Immunity. 1998;8:497–508.

Gay D, Saunders T, Camper S, Weigert M. Receptor editing: an approach by autoreactive B cells to escape tolerance. J Exp Med. 1993;177:999–1008.

Goodnow CC, Crosbie J, Adelstein S, Lavoie TB, Smith-Gill SJ, Brink RA, Pritchard-Briscoe H, Wotherspoon JS, Loblay RH, Raphael K, et al. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature. 1988;334:676–82.

Hartley SB, Crosbie J, Brink R, Kantor AB, Basten A, Goodnow CC. Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. Nature. 1991;353:765–9.

Hippen KL, Schram BR, Tze LE, Pape KA, Jenkins MK, Behrens TW. In vivo assessment of the relative contributions of deletion, anergy, and editing to B cell self-tolerance. J Immunol. 2005;175:909–16.

Nemazee DA, Burki K. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. Nature. 1989;337:562–6.

O'Keefe TL, Williams GT, Batista FD, Neuberger MS. Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies. J Exp Med. 1999;189:1307–13.

Pao LI, Lam KP, Henderson JM, Kutok JL, Alimzhanov M, Nitschke L, Thomas ML, Neel BG, Rajewsky K. B cell-specific deletion of protein-tyrosine phosphatase Shp1 promotes B-1a cell development and causes systemic autoimmunity. Immunity. 2007;27:35–48.

Pelanda R, Schwers S, Sonoda E, Torres RM, Nemazee D, Rajewsky K. Receptor editing in a transgenic mouse model: site, efficiency, and role in B cell tolerance and antibody diversification. Immunity. 1997;7:765–75.

Pillai S, Cariappa A, Pirnie SP. Esterases and autoimmunity: the sialic acid acetylesterase pathway and the regulation of peripheral B cell tolerance. Trends Immunol. 2009;30:488–93.

Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone-marrow B cells. J Exp Med. 1993;177:1009–20.

Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science. 2003;301:1374–7.

B Cell Trafficking

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Synonyms

B cell homing; B cell migration; B cell retention; B cell trafficking

Definition

B cell trafficking involves network interactions of well-controlled events among chemokines/chemokine receptors, their downstream G protein signals, and site-specific adhesion molecules. This enables migration of different stages of B cells from one compartment to another, followed by their retention in a specific niche to facilitate their development, maintenance, and execution of functions.

Overview

B cell trafficking plays a central role not only in B cell physiologic and pathologic effector functions, but it also orchestrates B cell development. B cell development occurs after precursors move from one specific niche and location to another. These niches and locations result in B cell development into well-defined stages with specific functional properties. For example, B cell maturation initially occurs in the bone marrow, which exports B cells to the secondary lymphoid organs (lymph nodes (LN), spleen, and other immune sites). There, through migration, retention, and thus interactions with other immune cells, including macrophages and T cells, B cells develop through well-defined stages in specific immune compartments. After development in secondary lymphoid organs, B cells undergo controlled release or egress. In the case of long-lived plasma cells, B cells migrate back to special niches in the bone marrow where they produce antibodies including autoantibodies in the case of autoimmunity. B cells can also develop into memory B cells which egress from secondary lymphoid organs and home to sites of autoimmune inflammatory sites such as the synovium (rheumatoid arthritis), skin (psoriasis), thyroid gland (thyroiditis), salivary gland (Sjögren's syndrome), pancreas (diabetes), granulomatous tissue ($ANCA^+$ vasculitis), and brain (multiple sclerosis) (Coca and Sanz 2012). For each stage of development, specific chemokines or adhesion molecules are produced to either attract or retain the B cells at these sites or, once development has occurred, are downregulated to enable migration of the B cells to a subsequent stage of development. This entry emphasizes mechanisms of B cell migration and chemokine receptor desensitization that results in development of spontaneous germinal centers (GCs) in the spleen during autoimmune diseases. Although autoantibody production in the spleen is most relevant to development of autoimmune disease, this entry includes insights from studies of GC development in both spleen and LN, which has many similarities. Furthermore, studies of B cell migration in LN are important to consider since, due to their ectopic location, direct visualization of B cell migration has been achieved and has provided important insights into GC development. A key difference is that the LN lacks marginal zone and marginal zone B cells, which play an important role in trapping apoptotic cells and transport of autoantigens to the GC in the spleen (Li et al. 2013). Current technology does not enable comprehensive studies of B cell migration in humans. Therefore, this review summarizes B cell migration derived primarily from mouse models, including autoimmune models. Although human marginal zone B cells can recirculate whereas rodent marginal zone B cells are sessile, studies have shown that the

cellular and molecular mechanisms of human B cell migration largely parallel what has been found in the mouse. This entry describes three key sites of B cell localization, the bone marrow, spleen, and peripheral tissues as well as the adhesion molecules and chemokines that enable the localization and development of B cells at these specific locations.

Bone Marrow

The bone marrow is the major organ for development and early maturation of B cells. B cells are generated from common lymphoid precursors (CLP) in the bone marrow before they egress into peripheral blood to reach secondary lymphoid organs. Specific stromal cells niches promote B cell development by signaling through cKit (CD117) and IL-7R (CD127). CXC-chemokine ligand 12 (CXCL12), also known as stromal cell-derived factor 1 (SDF-1), plays a role in pre-pro-B cells and less in pro-B cells B cell development (Hardy et al. 2007). The importance of the CXCR4/CXCL12 in B cell development became apparent in 1996 with the discovery that CXCL12 can attract early-stage B cell precursors via the chemokine receptor CXCR4 and have a key role of B cell lymphopoiesis. Regulation of CXCR4/CXCL12 has also been extensively analyzed due to its central role in regulation of cancer metastasis. The bone marrow is also the site to which terminally differentiated antibody-producing plasma cells home to and are maintained through the CXCR4/CXCL12.

An important goal for treatment of autoimmune disease is to deplete or limit the production of autoantibody-producing plasma cells. Bone marrow plasma cells do not express markers such as CD20 and, therefore, cannot be depleted by anti-CD20 therapies (Rituximab, Rituxan) (Coca and Sanz 2012). Other therapies including anti-CD22 (Epratuzumab) targets B cells and results in modulation of B cell function and migration, as CD22 regulates adhesion and inhibits B cell receptor (BCR) signaling. However, CD22 is not expressed by plasma cells or memory B cells (Ding et al. 2008). Depletion of plasma cells can be potentially achieved via blocking the homing and interactions of these cells with the supporting stromal cells. The persistence of plasma cells in the bone marrow is supported by soluble factors in cell-cell contact in the bone marrow microenvironment. Stromal cells provide growth factors such as IL-6 and cell contact-dependent signals, including very late antigen 4 (VLA4 or CD49d). Plasma cells also express other surface markers related to cell adhesion, rolling, tethering, and migration including integrin lymphocyte function-associated antigen 1 (LFA-1: also known as αLβ2 integrin or CD11a) and CD44 (Underhill et al. 2002). Co-blockade of LFA-1 and VLA-4 adhesion molecules has been shown to temporarily deplete long-lived plasma cells from the bone marrow. In addition, IgG plasma cells exhibit a unique property to interact specifically with E-selectin but not P-selectin despite the expression of P-selectin glycoprotein ligand 1 (PSGL-1). Such specificity has been found to be associated with the expression of alpha1,3-fucosyltransferase-VII (*Fut7*), but the selective downregulation of core 2 beta1-6-*N*-glucosaminyltransferase (Underhill et al. 2002).

Entry of B Cells to Secondary Lymphoid Organs Through High Endothelial Venules (HEV)

The interaction of lymphocytes with high endothelial venules (HEV) has been extensively studied in LN (Girard et al. 2012). B cell entry in LN is initiated by the lymphocyte homing receptor L-selectin (also known as CD62L), which mediates tethering and rolling of lymphocytes along HEV walls (Butcher and Picker 1996). The L-selectin recognizes a family of mucin glycoproteins, which are heavily sulfated fucosylated, expressed by HEV endothelial cells of peripheral lymph nodes, such as CD34 and the Lewis x antigen. HEV-mediated entry of naïve B cells into LN occurs through a multi-step adhesion and migration cascade. The lymphocyte rolling on the HEV cell walls first undergoes chemokine-induced activation of the integrin

LFA-1, which mediates lymphocyte arrest (sticking) on the HEV endothelium. LFA-1, which binds intercellular adhesion molecule 1 (ICAM-1 and ICAM-2) on endothelial cells, is a major integrin for B cell arrest in HEVs of peripheral lymph nodes. After crossing the HEVs, T and B cells migration follows different signals. T cells migrate along CCL19/21 chemokine-expressing fibroreticular cells (FRCs) using their prominently expressed CCR7 chemokine receptor to access the LN deep cortex, whereas B cells rely on their prominent CXCR5 expression to access the LN follicle (Reif et al. 2002). Newly resident B cells migrate toward the follicle centers, sites of high CXCL13 (the CXCR5 ligand) expression, whereas long-term LN follicle residents move toward the edges closer to egress sites.

Positioning within the Spleen or Lymph Node, Central Role of Desensitization of Chemokine Signaling

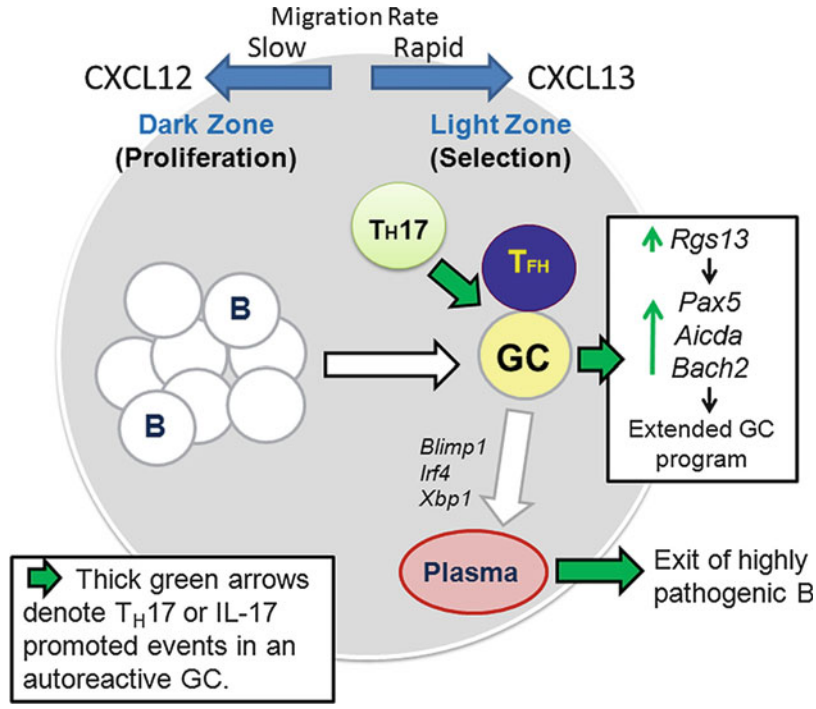
In the spleen, afferent lymphatics enter the marginal sinus as a source of antigen that interacts with B cells, especially marginal zone B cells, but newly formed transitional T1 B cells mostly enter through the central artery of the spleen through initial adhesion molecule interactions followed by chemokine gradients. During follicle development, B cells crawl on a fibroblastic reticular cell (FRC) network to reach B cell follicles, using CXCL13 and CXCR5 signaling (Pillai and Cariappa 2009). During the migration in the spleen, B cells upregulate the expression of a receptor for Sphingosine-1-phosphate (S1P), and after some time, they desensitize their chemokine receptors CXCR5 to sense the S1P-mediated egress signal at the edge of the follicles (Cinamon et al. 2004). Full development of marginal zone B cell precursors into marginal zone (MZ) B cells in the spleen does not occur until B cells reach the marginal zone (Pillai and Cariappa 2009). It is known that follicular (FO) B cell recirculate, whereas MZ B cells are sessile in that they are strategically positioned at the interface of the white pulp lymphoid follicles and red pulp cords in mouse spleen. Thus, once B cells enter the spleen marginal zone, expression of LFA-1 and $\alpha 4\beta 1$ by MZ B cells enables capture of MZ B cells by ICAM-1 and VCAM-1 expressed by resident stromal cells (Lu and Cyster 2002), leading to stabilization and maintenance of MZ B cells. Interestingly, MZ B cells produce lymphotoxin, which is a critical cytokine to induce the expression of ICAM-1 and VCAM-1 (Lu and Cyster 2002). Thus, MZ B cells also exhibit a unique property to maintain the integrity of MZ barrier in the spleen.

Role of Type I Interferons

Type I interferons (IFNs) are is primarily produced by plasmacytoid dendritic cells and they exhibit a unique mechanism to desensitize MZ B cells to S1P. High levels of Type I IFNs have been associated with autoimmunity, including systemic lupus erythematosus. Although Type I IFNs are frequently associated with B cell activation and maturation, they can also act on B cells in the marginal zone and possibly on the T2 to T3 transition B cells to induce their migration away from the marginal zone and into the spleen follicles. Type I IFNs can upregulate CD69, which leads to the downregulation of S1PR1 signaling, resulting in lack of localization of MZ B cells in the marginal zone. In addition, this can result in B cell capture of antigen in the marginal zone of the spleen and translocation of B cell carrying antigen into the follicle. In autoimmunity, such antigen-transporting B cells have been characterized as marginal zone precursor B cells, which also express high levels of Class II MHC, CD80, and CD86 and are therefore potent antigen-presenting cells (Wang et al. 2010).

Regulation of Migration within the Germinal Center

The cardinal feature of autoimmunity is the development of pathogenic autoantibodies, which occurs through tolerance loss in a germinal center reaction. Uncleared apoptotic cells coming into the spleen marginal zone can be transported into the spleen follicles by shuttling B cells to the GC light zone follicular dendritic cell (FDC) network region in autoimmune mice (Li et al. 2013). These B cells bearing



B Cell Trafficking, Fig. 1 IL-17-promoted GC production of pathogenic autoantibodies (Figure provided by John D. Mountz and Hui-Chen Hsu)

apoptotic cell antigens also exhibit the property to stimulate $CD4^+$ T cells (Li et al. 2013). Deposits of antigens or immune complexes in the FDC network and activation of $CD4^+$ T cells are important for the initiation of a GC response. Interestingly, the structure of spontaneous GC developed by autoimmune mice is very similar to that of GC developed after immunization, in that they are polarized into a FDC-enriched light zone and a B cell densely packed dark zone. In immunized GCs, intravital two-photon imaging revealed that B cells undergo interzonal migration between the light zone and the dark zone (Fig. 1). CXCL13-producing FDCs attract CXCR5-expressing follicular helper T (T_{FH}) cells as well as CXCR5-expressing B cells into what is known as the light zone of the GC for selection and affinity maturation, whereas CXCL12-producing stromal cells attract $CXCR4^+$ B cells to migrate to the GC dark zone for proliferation. It is further observed that, within the GC, B cell migration from dark zone to the light zone is much faster as compared to migration from the light zone to the dark zone (Victora et al. 2010). Such observation is consistent with the importance of B cell selection that takes place in the light zone where GC B cells must compete for antigen provided by immune complex deposits on FDCS as well as cytokines and stimulatory signals provided by T_{FH} cells to ensure their selection and survival. This suggests an important concept that within the GC light zone and dark zone compartments, there are additional signaling events that can regulate the arrest and thus the tempo of GC B cell migration.

Chemokine receptors are G protein-coupled receptors. One critical mechanism for desensitization of GC B cell migration response has been identified to be a class of GTPase-accelerating proteins called "regulator of G protein signaling (RGS)" (Druey 2009). RGS proteins can rapidly switch off G protein-coupled receptor signaling pathway by promoting GTP hydrolysis by the alpha subunit of heterotrimeric G proteins. In the GCs, RGS1, RGS13, and RGS16 are the critical RGS proteins that can switch off lymphocyte migration responses to CXCL12 and

CXCL13 (Mountz et al. 2011). Thus, exogenous factors that can upregulate the upregulation of RGS proteins can be critical to regulate GC B cell somatic hypermutation, class-switch recombination, affinity maturation, and selection process.

In a spontaneous autoimmune GC, IL-17-producing $CD4^+$ T cells (T_H17) upregulate RGS13 and RGS16 in B cells through the classical NF-κB pathway. This results in the dephosphorylation or inactivation of the G protein signaling pathway, thus making the B cell less responsive to CXCL12 and CXCL13 (Hsu et al. 2008; Xie et al. 2010). This migration arrest of GC B cells plays a role to enhance GC B cell maturation via extended interactions between GC B cells and $CD4^+$ T cells. In addition, this process sequesters B cells in GCs for a prolonged period of time through a process known as "delayed driven diversity," which enables upregulation of the GC-specific genes including *Aicda*, *Bach2*, and *Pax5* and downregulation of the plasma cell differentiation genes including *Blimp1*, *Irf4*, and *Xbp1* (Wang et al. 2013) (Fig. 1). Within the spontaneous autoimmune GCs, T_H17 cells have been found to be present mostly in the light zone, thereby promoting the necessary close contact between GC B cells and $CXCR5^+$ T_{FH} cells (Ding et al. 2013).

Control of Lymphocyte Egress from the Secondary Lymphoid Organs

S1P and its G protein-coupled receptor have been shown to be required for egress of both B and T cells from the spleen and LN. S1P was first implicated in the exit of lymphocytes from LN during homeostasis, when it was discovered that the immunosuppressive drug FTY720 (also known as Fingolimod), a potent inhibitory agonist of S1P receptors including S1P1 and S1P3, induced the sequestration of lymphocytes in the LN by inhibiting their egress into the lymph. Further analysis revealed that FTY720 acts by downregulating S1P1 signaling to prevent lymphocyte egress from the secondary lymphoid organs. S1P is generated in vivo by sphingosine kinases, particularly on red blood cells. S1P1 signaling appears to act principally by overcoming retention mediated by the Gαi-coupled receptors (CXCR5 and CCR7). FTY720 (Fingolimod, Gilenya) has been studied for applications to several autoimmune diseases including lupus and has become especially important as the first oral drug found to be highly efficacious in treatment of multiple sclerosis.

B Cell Migration to Peripheral Organs to Promote Autoimmunity as an Antigen-Presenting Cells

The above B cell migration focuses primarily on B cell development as antibody-producing cells and their homing back to the bone marrow to produce autoantibodies in autoimmunity. The role of B cells in inflammation was most dramatically demonstrated by the highly efficacious action of anti-CD20 that greatly ameliorate autoimmunity (lupus, RA) but do not greatly diminish autoantibodies production by plasma cells, which do not express CD20 (Coca and Sanz 2012). B cells are potent modulators of T cell responses by presenting antigen, providing co-stimulation, and secreting cytokines. B cell production of cytokines, including IFNγ, IL-6, and IL-4, can act as antigen-presenting cells as they express class II MHC, and express co-stimulatory molecules including CD80/86 and ICOS-L, and have been proposed to play a critical role in peripheral tissue inflammation. Furthermore, it is becoming clear that the different effector functions of B cells are tightly controlled and that these tasks are carried out by functionally and phenotypically distinct effector and regulatory B cell subsets. Regulatory B cells that produce IL-10 have been proposed to downregulate tissue inflammation. B cell migration to inflamed tissue is primarily mediated by similar mechanisms as found for their migration to peripheral lymphoid organs. A combination of adhesion molecules primarily selectins; integrins, including VCAM, ICAM/LFA-1; and chemokines, including CXCR4/CXCL12 and CXCR5/CXCL13, is thought to regulate migration of effector B cells to sites of inflammation (Stein and Nombela-Arrieta 2005).

Conclusion

Altered B cell migration can promote the development of autoimmunity and enhance T cell responses. Increased signaling and sensitivity of B cells within LN or spleen, as well as increased retention in the light zone with high activation-induced cytidine deaminase, result in autoantibody production and increased survival of autoantibody-producing B cells as plasma cells in bone marrow niches. Targeting of lymphocyte migration, including B cells, has been successful in treating autoimmune diseases, with anti-CD22 (Epratuzumab), which targets B cells and results in modulation of B cell function and migration and with the S1P inhibitor FTY720 (Fingolimod, Gilenya), which prevents egress of lymphocytes from lymphoid organs. Future therapies will include kinase inhibitors, which inhibit chemotaxis and migration primarily by inhibiting B cell activation, and specific chemokine or chemokine receptor blockade. Also, a key role for interferon in promoting B cell-driven germinal center responses by enabling translocation of antigen delivery B cells may be targeted through anti-interferon therapy.

Cross-References

- ▶ Cell Adhesion Molecules
- ▶ Systemic Lupus Erythematosus, Treatment

References

Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science. 1996;272(5258):60–6.

Cinamon G, Matloubian M, et al. Sphingosine 1-phosphate receptor 1 promotes B cell localization in the splenic marginal zone. Nat Immunol. 2004;5(7):713–20.

Coca A, Sanz I. Updates on B-cell immunotherapies for systemic lupus erythematosus and Sjogren's syndrome. Curr Opin Rheumatol. 2012;24(5):451–6.

Ding C, Foote S, et al. B-cell-targeted therapy for systemic lupus erythematosus: an update. BioDrugs. 2008;22(4):239–49.

Ding Y, Li J et al. IL-17RA is essential for optimal localization of follicular Th cells in the germinal center light zone to promote autoantibody-producing B cells. J Immunol. 2013;191(4):1614–1624.

Druey KM. Regulation of G-protein-coupled signaling pathways in allergic inflammation. Immunol Res. 2009;43(1–3):62–76.

Girard JP, Moussion C, et al. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. Nat Rev Immunol. 2012;12(11):762–73.

Hardy RR, Kincade PW, et al. The protean nature of cells in the B lymphocyte lineage. Immunity. 2007;26(6):703–14.

Hsu HC, Yang P, et al. Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. Nat Immunol. 2008;9(2):166–75.

Li H, Wu Q, et al. Cutting edge: defective follicular exclusion of apoptotic antigens due to marginal zone macrophage defects in autoimmune BXD2 mice. J Immunol. 2013;190(9):4465–9.

Lu TT, Cyster JG. Integrin-mediated long-term B cell retention in the splenic marginal zone. Science. 2002;297(5580):409–12.

Mountz JD, Wang JH, et al. Cytokine regulation of B-cell migratory behavior favors formation of germinal centers in autoimmune disease. Discov Med. 2011;11(56):76–85.

Pillai S, Cariappa A. The follicular versus marginal zone B lymphocyte cell fate decision. Nat Rev Immunol. 2009;9(11):767–77.

Reif K, Ekland EH, et al. Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. Nature. 2002;416(6876):94–9.

Stein JV, Nombela-Arrieta C. Chemokine control of lymphocyte trafficking: a general overview. Immunology. 2005;116(1):1–12.

Underhill GH, Minges Wols HA, et al. IgG plasma cells display a unique spectrum of leukocyte adhesion and homing molecules. Blood. 2002;99(8):2905–12.

Victora GD, Schwickert TA, et al. Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. Cell. 2010;143(4):592–605.

Wang JH, Li J, et al. Marginal zone precursor B cells as cellular agents for type I IFN-promoted antigen transport in autoimmunity. J Immunol. 2010;184(1):442–51.

Wang JH, New JS et al. Extension of the germinal center stage of B-cell development promotes autoantibodies in BXD2 mice. Arthritis & Rheum. 2013;65(10):2703–2712.

Xie S, Li J, et al. IL-17 activates the canonical NF-kappaB signaling pathway in autoimmune B cells of BXD2 mice to upregulate the expression of regulators of G-protein signaling 16. J Immunol. 2010;184(5):2289–96.

B1 Cells

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Synonyms

CD5 B cells; Leu-1 B cells; Ly-1 B cells; Lyt-1 B cells; T1 B cells

Definition

B1 cells constitute a small innate-like subpopulation of normal B lymphocytes whose most important role is constitutive secretion of protective (so-called natural) antibody.

Introduction

B cells are the terminal effectors of serological immunity and are responsible for the generation of life-preserving antibody in reaction to invasion by pathogenic microorganisms. This adaptive response develops over a period of days, and thus is not available at the time of initial infection; in fact, the delay in generation of adaptive immunity is the reason why immunizations to raise specific antibodies must occur well in advance of need. Beyond prior infection and immunization, however, the immune system is not devoid of early antibody protection. Preexisting, or "natural" antibody is provided by B1 cells. B1 cells secrete antibody constitutively and spontaneously, in the absence of exogenous stimulation, that recognizes many microbial determinants. In this way, B1 cells establish and maintain an initial antibody shield that defends against subsequent incipient infection. In recent years, additional functions have been ascribed to B1 cells that include housekeeping removal of cellular debris, as well as stimulation, polarization, and suppression of T cells.

History

Identification of B1 cells emerged from the initial finding over 30 years ago that malignant cells of mouse and human B cell leukemias and lymphomas express CD5. CD5 (formerly Ly-1 or Lyt-1 in the mouse system and Leu-1 or T1 in the human system) is a T cell surface antigen that would not be expected to be present on malignant cells of B cell origin. Further work revealed that CD5 expression is characteristic of a small subpopulation of normal B cells, as well as malignant B cells. Notably, CD5 expression in this subpopulation is low and B1 cell CD5 stains much less brightly with fluorochrome-conjugated anti-CD5 antibodies than CD5 on T cells, for which reason the recognition of $CD5^+$ B cells depends on flow cytometry with its use of photomultiplier detectors, rather than fluorescence microscopy. In mice, CD5 expression marks B cells that are phenotypically distinct from conventional (adaptive) B cells in expressing $IgM^{hi}IgD^{lo}CD45^{lo}CD23^{lo/-}CD43^+$ (plus $CD9^+$ shared with splenic marginal zone B cells and $CD11b^+$ for those $CD5^+$ B cells located in the peritoneal cavity). This subpopulation of B cells is now termed "B1" whereas the predominant population of conventional B cells is termed "B2." In mice, the B1 population is divided between B1a cells with the surface marker phenotype noted above, and B1b cells that share the same phenotypic markers ($IgM^{hi}IgD^{lo}CD45^{lo}CD23^{lo/-}CD43^+$) except that they are CD5 negative. B1a and B1b cells are regulated separately and behave somewhat differently, the latter sharing with B2 cells the capacity to respond to antigen stimulation in the form of vaccination or infection.

CD5 has been used in the past to define human B1 cells, and numerous early studies reported on the nature of human B1 cell antibodies and the activity of human B1 cells in various disease

states (Casali and Notkins 1989). However, in contrast to the situation in mice, CD5 is not a reliable marker for human B1 cells because it is promiscuously expressed by several different human B cell populations (including pre-naïve, transitional and activated B cells). Recent work indicates that the surface marker phenotype of human B1 cells is $CD20^+CD27^+CD43^+CD70^-$ (Griffin et al. 2011). Most of these human B1 cells express CD5, but the majority of $CD5^+$ human B cells do not express $CD20^+CD27^+CD43^+CD70^-$ and are not B1 cells.

Properties of Mouse B1 Cells: Natural Antibody

Mouse B1 cells have been accurately identified, isolated, and examined for decades whereas the new definition of human B1 cells is of much more recent vintage. Thus, much of what is known about B1 cells derives from studies of B1 cells in mice (Baumgarth 2011). This information points to critical roles for B1 cell-derived "natural" antibody in antimicrobial defense and housekeeping homeostasis, and potential roles in presentation of antigen and regulation of T cells.

Natural antibody is predominantly IgM and in mice, where the origin of antibodies can be accurately assessed, approximately 80–90 % of resting serum IgM, and approximately 50 % of resting serum IgA (the major isotype of switched B1 cell immunoglobulin) is generated by B1 cells. IgM is produced predominantly by B1 cells in the murine spleen (and bone marrow) that bear B1 cell surface markers and are not typical plasma cells. Although the highest concentration of mouse B1 cells is in the peritoneal cavity, peritoneal B1 cells produce only small amounts of antibody. Under certain activation conditions, however, B1 cells in the peritoneal cavity migrate to the spleen and there become high-secreting B1 cells. To an unknown extent, high-secreting splenic B1 cells may at times become quiescent and return to the peritoneal cavity as memory B1 cells.

Natural antibody produced by B1 cells tends to be polyreactive, autoreactive, and antimicrobial, at relatively modest affinity. B1 cell natural antibody is very effective in creating a line of defense against invasive pathogens – it has been shown for a number of microorganisms that recovery from experimental infection in mice requires the joint action of both B1 and B2 cell-derived antibodies (Baumgarth et al. 2000). It is accepted that preexisting B1 cell-derived antibodies counteract microbial activity during the lag period required for germinal center formation and B2 cell adaptive antibody production. In keeping with this, a substantial fraction of natural antibody recognizes phophorylcholine (PC), an invariant antigenic determinant of Gram-positive microbial membranes, including *S. pneumonia*, a major pathogen. However, anti-PC antibodies do not solely bind bacteria, as PC is present on autologous apoptotic cell membranes and oxidized lipids (Binder 2010). Another fraction of natural antibody recognizes phosphatidylcholine (PtC), a key constituent of senescent red blood cell membranes. These and other examples of autoreactivity have led to the concept that a second, parallel, function of B1 cell natural antibody lies in speeding the elimination of dead and dying cells and attendant cellular debris. In this way, potentially inflammatory and toxic molecules can be removed before damage in the form of immune cell priming or direct tissue injury can occur, thereby avoiding generation of adaptive autoantibody formation and instigation of destructive autoimmune disease (Kaveri et al. 2012). So natural antibody produced by mouse B1 cells fulfills two important functions: immediate defense against microbial pathogens and housekeeping removal of cellular and toxic debris.

The structure of mouse B1 cell natural antibody is distinctly more germline-like than B2 cell-derived adaptive antibody, by virtue of little or no somatic hypermutation and reduced N-region addition. This means that B1 cell antibodies more closely reflect $V_HD_HJ_H/V_LJ_L$ sequences as they are encoded in inherited DNA, in comparison with B2 cell antibodies.

In keeping with this, enforced N-addition in transgenic mice interferes with anti-pneumococcal defense, a substantial portion of which is provided by B1 cell germline-like antibodies. Because B1 cell antibodies tend to reflect sequences as encoded in the genome, and because B1 cell antibodies fulfill a crucial role in antimicrobial defense, it is considered that the B1 cell repertoire reflects Darwinian precepts such that sequences functioning to promote survival are retained. In this way, the B1 cell repertoire may be "tuned" over evolutionary time. In contrast, B2 cell antibodies that contain N-addition and undergo somatic mutation are selected on the basis of affinity, rather than functional efficacy. Together, B1 and B2 antibodies act in concert to thwart existential threats of an infectious nature.

A long-running controversy regarding the origin of mouse B1 cells has been resolved with the discovery of a distinct B1 cell progenitor ($CD45^{lo/-}CD19^{+}$), indicating that B1 cells constitute a separate and unique B cell lineage (Montecino-Rodriguez et al. 2006). Commitment to the B1 cell lineage appears to occur at a point in development even before the identified B1 cell progenitor, namely, at the common lymphocyte precursor (CLP) stage. Many adoptive transfer experiments have shown that fetal hematopoietic tissue (e.g., fetal liver) gives rise to B1 cells whereas adult hematopoietic tissue (e.g., bone marrow) largely does not. In keeping with this, B1 cells appear first in ontogeny, followed by B2 cells, which is the reason why B1 cells are "1" and B2 cells are "2" (Herzenberg 2000). In adult life, mouse B1 cells are thought to maintain themselves primarily through self-renewal, in which mature surface immunoglobulin-bearing B1 cells give rise to their own progeny, whereas B2 cells are continually replenished by stem cell differentiation. The ontologic switch from B1 cell to B2 cell generation occurs during the neonatal period and suggests "layering" of the B cell arm of the immune system in which a phylogenetically early, innate-type B cell population is overlain by a more recently evolved, adaptive-type B cell population. Because similar changes in T cell development (from γδ to αβ antigen receptors) and red blood cell development (from fetal to adult hemoglobin) occur in the same time frame, the B1/B2 cell dynamic may be part of a generalized ontological switch involving multiple hematopoietic lineages that occurs around the time of birth, and may be directed by the RNA-binding protein, Lin28b (Yuan et al. 2012).

Properties of Mouse B1 Cells: Immunoregulation

Beyond secretion of natural antibody, B1 cells affect other immune system constituents in both positive and negative ways. B1 cells strongly stimulate activation and proliferation of naïve T cells in allogeneic co-cultures, indicating that B1 cells efficiently present antigen. This depends, at least in part, on constitutively elevated expression of the co-stimulatory molecule, CD86, and, to a lesser extent, on elevated expression of CD80. CD86 expression joins several other characteristics of unstimulated B1 cells that are similar to features of stimulated B2 cells, a list that includes phosphorylated extracellular signal-regulated kinase (ERK), which is a key protein in B cell receptor signal transduction. Both constitutively elevated CD86 and phospho-ERK result from a state of chronic signaling in B1 cells that may be dictated by autoreactive specificities.

Beyond triggering T cell expansion, B1 cells can influence T cell function toward inflammation by inducing naïve $CD4^{+}$ T cells to differentiate into IL-17-expressing pro-inflammatoryTh17 cells. In contrast, B2 cells cannot induce Th17 cell differentiation unless stimulated first. Conversely, B1 cells can negatively influence T cell function through immunosuppression produced by secretion of IL-10, although B1 cells are not alone in the ability to produce IL-10 and, in addition, other means of immunosuppression have been described for B cells and other immune cells. Inasmuch as B1 cells can present antigen to T cells, can stimulate T cell expansion, can induce Th17 cell differentiation, and can suppress T cell activity, B cells

appear to be in a position to strongly influence the nature and direction of T cell responses, depending on whether stimulatory or suppressive activities dominate, although the extent to which this occurs in vivo has not been determined.

In addition to surface antigen expression, natural antibody secretion, chronic intracellular signaling, and T cell stimulation, differentiation, and suppression, B1 cells differ from B2 cells in a number of other respects that include overall size, viability in vitro, developmental requirements, signaling characteristics, and expression of a number of gene transcripts and proteins.

Properties of Human B1 Cells

The unsatisfactory nature of CD5 as the defining feature of human B1 cells impelled new efforts to characterize the B1 cell surface marker phenotype in humans. A new phenotypic definition of human B1 cells emerged from a "reverse engineering" approach, in which several functional features of mouse B1 cells – spontaneous secretion of IgM, chronic intracellular signaling, and efficient T cell stimulation – were combined as a set of qualifying criteria that any putative human B1 cell population should satisfy. Testing individual populations of human B cells expressing various combinations of surface markers revealed a small population of B cells (identified by CD20 rather than CD19 to avoid immunoglobulin-secreting plasmablasts that lose CD20 but maintain CD19) which co-expresses CD27 and CD43 and fulfills the functional criteria above, and is thus denoted B1 cells (Griffin et al. 2011). $CD20^+CD27^+CD43^+$ B1 cells are routinely found in umbilical cord blood, where CD43 expression more or less coincides with CD27 expression, and in adult peripheral blood, where CD43 identifies a subpopulation within the larger population of $CD27^+$ B cells. Because CD27 is an acknowledged marker for human memory B cells, identification of human B1 cells as a subpopulation of $CD27^+$ B cells indicates that what had been considered a homogeneous memory B cell population is actually heterogeneous by virtue of containing $CD27^+CD43^+$ constituents that are B1 cells. These results suggest that B1 cells should be removed prior to conducting studies on memory B cells defined by CD27 expression.

By definition, human B1 cells recapitulate three key B1 cell features identified in the mouse system, as noted above, because these are qualifying criteria for designation of B cells as "B1." In addition, $CD20^+CD27^+CD43^+$ B cells preferentially bind PC, and preferentially bind the DWEYS peptide mimetope of DNA, in comparison to naïve and memory B cells, mirroring the activities of mouse B1 cells in producing antimicrobial and autoreactive specificities and thereby supporting the phenotypic designation of human B1 cells. Human B1 cells also secrete immunosuppressive IL-10, establishing still another parallel with mouse B1 cells, although only a subset of human B1 cells functions this way. There is as yet no evidence relating human B1 cells and Th17 cell differentiation, so it is not known whether B1 cells share this role across species.

Enumeration of human B1 cells by flow cytometry (Kaminski et al. 2012) depends on a number of parameters that have not been standardized among laboratories. These include, among others, efficiency of antibody binding, choice of fluorophore reagents, sensitivity of fluorescence detection, hierarchy of gating strategy, and competency of computerized algorithms for data analysis. This is one reason, in addition to the genetic and environmental heterogeneity of the human population, why normal values for B1 cells in healthy individuals have not yet been established. However, it is generally considered that B1 cells constitute on the order of 1–9 % of circulating B cells, but may, on occasion, fall outside that range. Whereas mouse B1 cells are predominantly located at serosal surfaces, no reservoir of human B1 cells has been discovered and there is no evidence that human B1 cells are especially located in the peritoneum.

Recognition of human B1 cells requires special considerations. CD43 is an activation antigen and can be expressed by stimulated human naïve

and memory B cells. In the latter case, $CD20^+CD27^+$ memory B cells that acquire CD43 expression would recapitulate the marker profile of human B1 cells. However, stimulated human naïve and memory B cells that express CD43 also express CD69 and CD70 activation antigens whereas human B1 cells do not. To exclude putative stimulated memory B cells, the formal phenotypic characterization of human B1 cells has been expanded to include CD70-negativity, so that B1 cells are fully defined as $CD20^+CD27^+CD43^+CD70^-$ (Griffin et al. 2011).

B1 cell recognition can suffer from doublet formation. In peripheral blood, the number of T cells far outweighs the number of B cells, and most T cells express CD27 and CD43. Thus, any stochastic tendency of a naïve or memory B cell to form a doublet would likely produce a B:T combination in which the B cell contributes CD20 (or CD20 and CD27) and the T cell contributes CD27 and CD43 expression. In other words, B:T doublets containing naïve or memory B cells could masquerade as $CD20^+CD27^+CD43^+$ B1 cells, producing false-positive counts. Several methods can be utilized to exclude putative B cell-containing doublets either before or after acquisition of flow cytometric data (Griffin and Rothstein 2012).

Clinical Correlations

The precise roles of human B1 cells in generating natural antibody, in establishing antimicrobial defense, in fulfilling housekeeping homeostasis, and in regulating T cell activity have not been determined, despite suggestive evidence garnered from in vitro and in vivo assays. It is unknown whether human B1 cell-derived natural antibody functions to oppose or delay the onset of cancer, atherosclerosis, neurodegenerative disease, and/or other dyscrasias of aging, as suggested by recent studies (Kyaw et al. 2011; Rodriguez-Zhurbenko et al. 2013; Boutajangout et al. 2011). It is unknown to what extent human B1 cells are pathogenically connected to autoimmune diseases such as lupus and rheumatoid arthritis, as suggested by mouse models (Duan and Morel 2006). And it is unknown to what extent B1 cells are lost following B cell depletion therapy to treat autoimmunity, chronic lymphocytic leukemia (CLL), and lymphoma, or to what extent B1 cells are repopulated from adult stem cells after bone marrow transplantation. However, to the extent that B1 cells are responsible for serum natural antibody in *H. sapiens* as in *M. musculus*, clinical conditions of agammaglobulinemia and common variable immunodeficiency (CVID) are likely to be associated with abnormal loss or abnormal function of human B1 cells, in keeping with the severe derangement of baseline serum immunoglobulin levels in these conditions. The extent to which human B1 cells play a role in other clinical situations and immune dyscrasias remains to be elucidated.

Conclusion

B1 cells are small in number but large in function, in mice and presumably other species including our own. B1 cells are considered part of the innate immune system and produce natural antibody that is essential to defense against microbial infection and removal of cellular debris. B1 cells also stimulate, differentiate, and suppress T cells, although the extent to which this takes place in vivo has not been clarified. Human B1 cells were initially characterized as CD5 positive, but this definition has been supplanted by identification as a $CD43^+$ subpopulation of $CD27^+$ B cells. Although human B1 cells recapitulate many of the functions described for mouse B1 cells, the role and function of B1 cells in human health and disease remains to be determined.

Cross-References

- ▶ Autoantibodies in Rheumatoid Arthritis
- ▶ BCR Signaling
- ▶ CD5
- ▶ Regulatory B Cells
- ▶ Repertoire Selection

▶ Systemic Lupus Erythematosus, Autoantibodies
▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. Nat Rev Immunol. 2011;11:34–46.
Baumgarth N, Chen J, Herman OC, Jager GC, Herzenberg LA. The role of B-1 and B-2 cells in immune protection from influenza virus infection. Curr Top Microbiol Immunol. 2000;252:163–9.
Binder CJ. Natural IgM antibodies against oxidation-specific epitopes. J Clin Immunol. 2010;30 Suppl 1:S56–60.
Boutajangout A, Ingadottir J, Davies P, Sigurdsson EM. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. J Neurochem. 2011;118:658–67.
Casali P, Notkins AL. CD5+ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. Immunol Today. 1989;10:364–8.
Duan B, Morel L. Role of B-1a cells in autoimmunity. Autoimmun Rev. 2006;5:403–8.
Griffin DO, Rothstein TL. Human B1 cell frequency: isolation and analysis of human B1 cells. Front Immunol. 2012;3:122.
Griffin DO, Holodick NE, Rothstein TL. Human B1 cells in umbilical cord and adult peripheral blood express the novel phenotype CD20 + CD27 + CD43 + CD70. J Exp Med. 2011;208:67–80.
Herzenberg LA. B-1 cells: the lineage question revisited. Immunol Rev. 2000;175:9–22.
Kaminski DA, Wei C, Qian Y, Rosenberg AF, Sanz I. Advances in human B cell phenotypic profiling. Front Immunol. 2012;3:302.
Kaveri SV, Silverman GJ, Bayry J. Natural IgM in immune equilibrium and harnessing their therapeutic potential. J Immunol. 2012;188:939–45.
Kyaw T, Tay C, Krishnamurthi S, Kanellakis P, Agrotis A, Tipping P, Bobik A, Toh BH. B1a B Lymphocytes are atheroprotective by secreting natural IgM that increases IgM deposits and reduces necrotic cores in atherosclerotic lesions. Circ Res. 2011;109:830–40.
Montecino-Rodriguez E, Leathers H, Dorshkind K. Identification of a B-1 B cell-specified progenitor. Nat Immunol. 2006;7:293–301.
Rodriguez-Zhurbenko N, Martinez D, Blanco R, Rondon T, Grinan T, Hernandez AM. Human antibodies reactive to NeuGcGM3 ganglioside have cytotoxic antitumor properties. Eur J Immunol. 2013;43:826–37.
Yuan J, Nguyen CK, Liu X, Kanellopoulou C, Muljo SA. Lin28b reprograms adult bone marrow hematopoietic progenitors to mediate fetal-like lymphopoiesis. Science. 2012;335:1195–200.

B7 and CD28 Families

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Synonyms

B7-1; B7-2; B7-H3; B7-H4; B7S1; B7x; CD152; CD273; CD274; CD276; CD278; CD279; CD80; CD86; CTLA-4: CTL antigen-4; ICOS: inducible costimulator; ICOS-L: ICOS ligand; PD-1: programmed death-1; PD-L1 and PD-L2: PD-1 ligand 1 and 2

Definition

T cell costimulation is a signal required for the full activation of naïve T lymphocytes in the presence of T cell receptor signal. T cell coinhibition is a signal required for inhibition of activated T lymphocytes in the presence of T cell receptor signal.

Introduction

According to the two-signal model, optimal activation of a naïve T cell requires the simultaneous occurrence of two signals. Signal 1 is an antigen-specific signal generated by T cell receptor (TCR) recognition of peptide-MHC presented by an antigen-presenting cell (APC)(Bour-Jordan et al. 2011; Zang and Allison 2007). Signal 2, called costimulation, is an antigen-independent signal produced by the interaction between CD28 on T cells and ligand B7-1 (CD80) or B7-2 (CD86) on APC. Activation through the TCR (Signal 1) in the absence of costimulation (Signal 2) leads to functional inactivation or clonal deletion of T cells. After activation, T cells express CTLA-4 (CD152), a homolog of CD28. CTLA-4 also binds B7-1 and B7-2, which results in coinhibition attenuating T cell responses. T cell

B7 and CD28 Families, Table 1 Structure, expression, and function of the B7 ligand family and the CD28 receptor family

Ligands			Receptors and binding partners			Function
Name	Structure	Expression	Name	Structure	Expression	
B7-1 (CD80)		APC, activated T cells	CD28 CTLA-4 (CD152) PD-L1		T cells, Treg, NK activated T cells, Treg	Costimulation Coinhibition
B7-2 (CD86)		APC, activated Tcells	CD28 CTLA-4		T cells, Treg, NK activated Tcells, Treg	Costimulation Coinhibition
ICOS-L (CD275, B7h, B7RP-1, GL50, B7H2, LICOS)		APC, tissues	ICOS (CD278)		activated T cells, Treg	Costimulation
PD-L1 (CD274, B7-H1)		APC, T cells, tissues, tumors	PD-1 (CD279) B7-1		activated T and B cells, Treg myeloid cells, thymocytes	Coinhibition
PD-L2 (CD273, B7-DC)		DC, macrophages	PD-1		activated T and B cells, Treg myeloid cells, thymocytes	Coinhibition
B7-H3 (CD276)	 	DC, macrophages, tissues, activated T cells, tumors	unidentified receptors		activated T cells, NK	Costimulation Coinhibition
B7x (B7-H4, B7S1)		tissues, tumors	unidentified receptors		activated T cells	Coinhibition

costimulatory and coinhibitory pathways are essential orchestrators and regulators of the adaptive immune response. In recent years, the CD28 receptor and B7 ligand families have been expanded to include a total of four [CD28, CTLA-4, ICOS (CD278), and PD-1 (CD279)] and seven members [B7-1 (CD80), B7-2 (CD86), ICOS-L (CD275), PD-L1 (CD274), PD-L2 (CD273), B7-H3 (CD276), B7x (B7-H4 or B7S1)], respectively (Table 1).

The B7-1/B7-2/CD28/CTLA-4 Pathway

This pathway is the most extensively characterized T cell costimulatory and coinhibitory pathway. Ligands B7-1 and B7-2 have extracellular IgV-IgC domains, and it is the IgV domain that is responsible for receptor binding and dimerization (Chattopadhyay et al. 2009). Both B7-1 and B7-2 are mainly expressed on APC such as dendritic cells (DCs), macrophages, and B cells, but their expression kinetics differ. APC activation is necessary for induction of B7-1 expression, whereas B7-2 is constitutively expressed on resting APC in low levels and is enhanced upon APC activation. Similarly, B7 receptors CD28 and CTL antigen-4 (CTLA-4) are both expressed on T cells, but differ in their expression kinetics as well. CD28 is constitutively expressed on naïve and activated T cells, whereas CTLA-4 expression is induced in response to TCR signal, so it is detectable on activated T cells and regulatory T cells (Treg) but not on naïve T cells. Both CD28 and CTLA-4 have an extracellular IgV domain, but they differ markedly in their localization in T cells (Rudd et al. 2009). CD28 is localized on the cell surface. By contrast, the majority of CTLA-4 is found in intracellular compartments such as the trans-Golgi network, endosomes, and lysosomes. Therefore, factors that regulate CTLA-4 protein access to the cell surface can spatially and temporally determine the extent to which CTLA-4

regulates T cell function. Although B7-1 and B7-2 can bind both CD28 and CTLA-4 via the same MYPPPY motif, the affinity of CTLA-4 for these ligands is much higher compared to that of CD28. Clearly, expression kinetics, location, and binding affinity are diverse among the ligands and receptors in this pathway.

In the presence of TCR signal, CD28 co-localizes with TCR in the central region of the immunological synapse, where the interaction between B7-1/B7-2 on APC and CD28 on T cells leads to costimulation. The costimulatory pathway not only amplifies phosphorylation of TCR-dependent kinases but also establishes a distinct signaling and transcriptional program (Rudd et al. 2009). CD28-mediated costimulation can also drive the formation of a mature immunological synapse partially via the recruitment of lipid rafts to the synapse. The cytoplasmic tail of CD28 contains several motifs responsible for binding of signaling molecules. The proximal motif can be phosphorylated by Src family kinases and then binds PI3K, Grb2, and GADS; the distal motif can be phosphorylated by Lck and Fyn kinases and then binds Grb2 and Filamin-A; a proline-rich region is associated with Itk binding. One of the most important functions of CD28-mediated costimulation is to markedly promote the production of T cell growth factor IL-2 and the expression of the high-affinity IL-2 receptor, which is pivotal for optimal clonal expansion of naïve T cells. CD28-mediated costimulation also controls T cell survival by enhancing expression of the anti-apoptotic factor Bcl-X_L. Finally, CD28-mediated costimulation is able to increase glucose uptake and glycolysis in order to meet the elevated metabolic requirement following T cell activation. B7-1/B7-2/CD28-mediated costimulation is essential for the initial activation of naïve T cells, while effector and memory T cells are less dependent on this pathway to maintain their function. Most normal tissue cells do not express B7-1 or B7-2, and immature APC express only low levels of B7-2, so these cells cannot provide enough ligands to bind CD28 on naïve T cells that may recognize MHC-peptide complex presented by these cells. The consequence is that these tissue cells will not fully activate the naïve T cells they bind, but rather, results in anergy, functional inactivation of T cells. This is one of the fundamental mechanisms the immune system has to maintain self-tolerance and prevent autoimmune responses against self-tissues.

In contrast to the costimulatory activity of CD28, the interaction of B7-1 or B7-2 with CTLA-4 is essential for limiting the proliferative response of activated T cells to antigen and CD28-mediated costimulation (Egen et al. 2002). The importance of CTLA-4 function is evidenced by the fact that *CTLA-4* gene knockout mice develop a lethal lymphoproliferative disorder and human *CTLA-4* gene polymorphisms are strongly linked with some autoimmune diseases (Scandiuzzi et al. 2011). T cell coinhibition by CTLA-4 is achieved mainly through two mechanisms: competition for ligands and induction of an inhibitory signal. Like CD28, CTLA-4 also moves to the immunological synapse, but its accumulation in the immunological synapse is proportional to the strength of the TCR signal (Egen et al. 2002). At the immunological synapse, CTLA-4 can compete with CD28 for B7-1/B7-2 engagement. Because CTLA-4 has greater affinity for B7 ligands than does CD28, CTLA-4 can sequester B7-1/B7-2 molecules, which in turn reduces CD28-dependent costimulation. Crystal structures reveal that CTLA-4 and B7 pack in a strikingly periodic arrangement in which bivalent CTLA-4 homodimers bridge bivalent B7 homodimers, therefore allowing the formation of a zipper-like protein lattice that could, in theory, assemble in the immunological synapse to compete with CD28 and/or to initiate inhibitory signal (Chattopadhyay et al. 2009; Schwartz et al. 2002). CTLA-4 can induce an inhibitory signal through its cytoplasmic tail that is 100 % conserved among mammalian species. The cytoplasmic tail of CTLA-4 contains two tyrosine residues that can be phosphorylated and subsequently recruit phosphatases like SH2 domain-containing phosphatase-2 (SHP-2). These phosphatases then dephosphorylate TCR-ζ as well as other components of the TCR proximal signal molecules such as linker for activation of

T cells (LAT), tyrosine kinases Fyn and Lck, and ζ chain-associated protein kinase of 70 kDa (ZAP-70). CTLA-4's cytoplasmic tail also contains a lysine-rich motif which can be bound by the serine/threonine protein phosphatase 2A (PP2A) resulting in thereby repression of CTLA-4 inhibitory function. In addition, CTLA-4 can inhibit the CD28 signal. Some signaling molecules such as PI3K and PP2A are able to bind cytoplasmic tails of both CTLA-4 and CD28, and CTLA-4 may sequester these molecules thereby reducing availability to induce CD28-dependent costimulation. CTLA-4 may also bring phosphatases in proximity to CD28's cytoplasmic tail, thereby repressing CD28-mediated costimulation. Although the precise nature of the signals transmitted through CTLA-4 remains controversial, it is likely that CTLA-4 can antagonize both TCR-dependent and CD-28-dependent signals (Rudd et al. 2009). In addition to activation of naïve T cells, B7/CD28 interactions are needed for the development and maintenance of regulatory T cells (Treg). Treg constitutively express high levels of CTLA-4 on their surface, and CTLA-4 may have an important cell-autonomous inhibitory function in Treg. In summary, the pathway of B7-1/B7-2/CD28/CTLA-4 has critical roles in naïve T cell activation and Treg homeostasis.

The ICOS-L/ICOS Pathway

ICOS (inducible costimulator, CD278) is the third member of the CD28 family (Nurieva et al. 2011; Zang and Allison 2007). Unlike CD28, ICOS is not expressed on naïve T cells but is induced after T cell activation. Both TCR and CD28 signals enhance ICOS expression, whereas the RING-type ubiquitin ligase family member Roquin mediates degradation of ICOS mRNA. ICOS-L (also called B7h, B7RP-1, GL50, B7H2, and LICOS) is the ligand for ICOS and is constitutively expressed on B cells, macrophages, and DCs and can be induced in nonlymphoid tissues by inflammatory stimuli. ICOS-L expression on B cells is downregulated by signaling through the B cell receptor and IL-4 receptor or after interaction with ICOS as a negative feedback loop.

The cytoplasmic tail of ICOS has an YMFM motif that, after tyrosine phosphorylation, binds to the P85α and P50α subunits of PI3K. Compared to CD28, ICOS stimulates greater PI3K activity and a concomitant increase in Akt signal. However, unlike CD28, the ICOS YMFM motif does not bind Grbs which is critical for IL-2 production. Therefore, ICOS engagement has little effect on IL-2 secretion, but increases production of IL-4, IL-5, IL-6, IL-10, interferon-γ (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) by T cells. The ICOS signal promotes T cell activation, differentiation, and effector responses.

The ICOS-L/ICOS pathway provides critical T cell help to B cells. Chemokine CXC motif receptor 5 (CXCR5)-positive follicular helper CD4 T (Tfh) cells are a unique T cell subset which provide help to B cells and promote the formation of long-lived antibody responses, and ICOS-L engagement of ICOS on CD4 T cells provides signals required for initiation and maintenance of Tfh differentiation (Crotty 2011). At the time of DC priming, the expression of ICOS on CD4 T cells is required for the expression of transcription factor Bcl6, a master regulator of Tfh differentiation. After priming, $Bcl6^{+}CXCR5^{+}$ Tfh cells express an elevated level of ICOS and migrate to the T-B border where the B cell-dependent phase of Tfh differentiation occurs. B cell-expressed ICOS-L and Tfh-cell expressed ICOS are required for the production of IL-21, a cytokine for B cell maturation, as well as the formation of a germinal center. Due to the vital role of the ICOS-L/ICOS in Tfh generation and promotion of humoral immunity, deficiency of ICOS or ICOS-L results in substantially reduced numbers of Tfh cells, impaired germinal center formation, and dysfunctional isotype switching.

ICOS deficiency accounts for the full spectrum of manifestations in a proportion of patients with common variable immunodeficiency (CVID) (Scandiuzzi et al. 2011; Yong et al. 2009). Specifically, a homozygous partial deletion of the *ICOS* gene in some CVID

patients results in deficiency in the expression of ICOS protein on activated T cells. As a consequence, the patients cannot generate or sustain normal numbers of memory B cells and have markedly reduced levels of serum IgG and IgA. Even after vaccination these patients lack detectable amounts of specific IgG. The genetic linkages between ICOS deletion and the clinical features of CVID emphasize the critical role of the ICOS-L/ICOS pathway in antibody production.

The PD-L1/PD-L2/PD-1 Pathway

PD-1 (programmed death-1, CD279) is a member of the CD28 family (Keir et al. 2008; Okazaki and Honjo 2007). The extracellular part of PD-1 contains a single IgV domain but lacks the membrane proximal cysteine that mediates the interchain disulfide bond, so it is monomeric on the cell surface (Chattopadhyay et al. 2009). PD-1 is expressed during thymic development but is absent on mature naïve T cells. In contrast to the restricted expression of other CD28 family members to T cells, PD-1 is induced on T cells, B cells, and some myeloid cells after activation. PD-1 has two ligands, PD-L1 (B7-H1, CD274), which also binds B7-1, and PD-L2 (B7-DC, CD273). PD-L1 is widely expressed on hematopoietic cells including APC and on several parenchymal tissues including the vascular endothelium and epithelium of several organs, whereas PD-L2 expression is mostly restricted to DCs and macrophages. IFN-γ is a major regulator of PD-L1 expression for a wide range of cell types (Barach et al. 2011). The different expression patterns of the PD-1 ligands may relate to their distinct functional abilities. Although both ligands have extracellular IgV-IgC domains and PD-L2 has greater affinity for PD-1 than PD-L1, PD-L1 is usually more effective than PD-L2 at triggering PD-1-mediated T cell inhibition.

The PD-L/PD-1 pathway plays an important role in the control of tolerance and autoimmunity (Sharpe et al. 2007), which was first revealed when it was observed that *PD-1* gene knockout mice slowly developed spontaneous autoimmune diseases. PD-1 engagement during TCR signaling can inhibit T cell proliferation, cytokine production, cytolytic function, and T cell survival. PD-1 is more effective at attenuating weak TCR signals than strong ones. Unlike CTLA-4, PD-1 function solely depends on its cytoplasmic tail which contains an immunoreceptor tyrosine-based switch motif (ITSM) (Okazaki and Honjo 2007; Sharpe et al. 2007). Ligation of PD-1 and TCR leads to tyrosine phosphorylation of the ITSM which can then be bound by SHP-1 and SHP-2; these phosphatases can dephosphorylate TCR-associated CD-3ζ and ZAP-70, resulting in inhibition of PI3K and downstream Akt. In contrast, CTLA-4 inhibits Akt activation but does not alter PI3K activity. PD-1 ultimately decreases induction of cytokines and cell survival proteins. During autoimmune responses, this pathway limits the initial phase of activation and expansion of self-reactive T cells and restricts self-reactive effector T cells and targets organ damage.

The PD-L1/PD-1 pathway contributes critically to T cell exhaustion and viral persistence during some chronic infections (Hofmeyer et al. 2011). Cytotoxic CD8 T lymphocytes (CTLs) play a pivotal role in the control of infection by killing infected cells. During chronic viral infection, the persistent presentation of antigen causes highly upregulated PD-1 on CTLs and upregulated PD-L1 on APC or resident tissue cells. As a consequence, activated CTLs become exhausted, lose effector function, and are unable to eliminate infection. Therefore, the PD-L1/PD-1 pathway is emerging as one of major regulators converting effector CTLs into exhausted CTLs during chronic infection with human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and other pathogens capable of establishing chronic infections.

The B7-H3 and B7x Pathways

B7-H3 (CD276) and B7x (B7-H4 or B7S1) are the most recently discovered members of the B7 family, and their contribution to immune

response has not yet been clearly defined. Furthermore, the receptors for B7-H3 and B7x are currently unidentified.

B7-H3 has two major isoforms. Mouse B7-H3 contains extracellular IgV-IgC domains, whereas human B7-H3 has tandemly duplicated IgV-IgC-IgV-IgC domains because of exon duplication (Collins et al. 2005). However, no functional difference has been observed between these two isoforms. B7-H3 protein is induced on APC, NK cells, T cells, fibroblasts, fibroblast-like synoviocytes, and some epithelial cells. microRNA-29 represses B7-H3 translation. The physiological function of B7-H3 on T cell proliferation and cytokine production remains controversial (Hofmeyer et al. 2008); B7-H3 binds activated T cells, leading to costimulation in some cases and to coinhibition in others. Similarly, studies in *B7-H3* gene knockout mice support a costimulatory role in some disease models and a coinhibitory role in others. The function of B7-H3 in cancer immune responses is also controversial (Barach et al. 2011; Zou and Chen 2008). In some murine models of cancer, expression of B7-H3 on cancer cells actives tumor-specific CTLs and slows tumor growth. In contrast to mouse tumor studies, the majority of studies with human cancers demonstrate that patients with strong B7-H3 expression on cancer cells are more likely to have poor clinical outcome, suggesting a coinhibitory role. It remains to be determined whether the contrasting roles of B7-H3 in T cell function are attributed to multiple receptors.

B7x, like most other B7 family members, is composed of extracellular IgV-IgC domains linked by a transmembrane region to a very short cytoplasmic tail. B7x mRNA is broadly expressed across a wide range of organs but exhibits an expression pattern inverse of that of B7-2 mRNA, with higher B7x mRNA expression in peripheral nonlymphoid tissues and very low expression in the hematopoietic compartment. B7x protein is hardly detectable on immune cells even after culture in various stimulating, suppressive, and maturation conditions, but is found on epithelial cells of some organs and β cells of the pancreas. B7x inhibits both CD4 and CD8 T cell proliferation and cytokine production in vitro (Zang and Allison 2007; Zou and Chen 2008). The combination of the in vivo expression pattern and the in vitro T cell coinhibitory capability of B7x suggests that the B7x pathway may be important in regulating tolerance and autoimmunity in nonlymphoid organs. The in vivo function of the B7 pathway, however, remains largely unknown. Although B7x protein expression is rare in healthy tissues, it is abundant in human malignancy (Barach et al. 2011), including cancers of the prostate, ovary, brain, lung, breast, kidney, pancreas, gut, esophagus, uterus, and skin. Importantly, overexpression of B7x in cancer often correlates with disease progression and poor clinical outcome, suggesting the B7x pathway may be exploited by cancer cells to evade antitumor immune responses.

Drugs Developed Based on the B7 and CD28 Families

The fundamental importance of costimulatory and coinhibitory signals for lymphocyte activation have spurred a large amount of effort in developing new immunotherapies by manipulating the pathways of the B7 and CD28 families. In 2005, the US Food and Drug Administration (FDA) approved Abatacept (CTLA-4-Ig) for the treatment of adult rheumatoid arthritis; a few years later, its use was extended to juvenile idiopathic arthritis (Felix et al. 2010). Abatacept is a fusion protein consisting of the extracellular domain of human CTLA-4 and the Fc portion of human IgG1, which has been modified and does not bind complement nor the Fc receptors. Compared to CD28, CTLA-4 has much higher affinity for B7-1 and B7-2; therefore, Abatacept can hamper B7-1/B7-2/CD28-mediated costimulation, resulting in increased cell death, anergy induction, and blockade of cell differentiation of T cells. Belatacept, the second generation of Abatacept, has higher affinity and is being tested in clinical trials for use in organ transplantation. In 2011, the US

FDA approved ipilimumab for the treatment of metastatic melanoma (Sharma et al. 2011). Ipilimumab, a human monoclonal antibody against CTLA-4, functions to block the interaction of CTLA-4 with B7-1 and B7-2, while leaving TCR and CD28 signals intact, which results in increased T cell function against cancer cells. Ipilimumab is currently in clinical trials in patients with other types of cancers. Clearly, research on T cell costimulation and coinhibition holds promise for the development of novel therapies and diagnoses.

Conclusion

Costimulation and coinhibition control appropriate T cell activation, proliferation, differentiation to effector function, and memory cell generation. The intense efforts towards understanding B7 and CD28 molecules over the past two decades have shaped much of our understanding regarding the immune system and disease mechanisms. The B7-1/B7-2/CD28/CTLA-4 pathway serves as the main switch regulating the clonal expansion of activated naive T cells as well as Treg homeostasis; the ICOS-L/ICOS pathway provides critical T cell help to B cells and germinal center formation; the PD-L1/PD-L2/PD-1 pathway controls effector T cell function and T cell exhaustion. PD-L1, B7-H3, and B7x are expressed in the peripheral tissues and may therefore act as gatekeepers for immune cells in nonlymphoid organs to prevent self-attack, whereas cancer and chronic infectious pathogens exploit these pathways to achieve immune evasion. While further studies are required to better understand the molecular mechanisms of these pathways and their particular roles in various diseases, the pathways of T cell costimulation and coinhibition are promising drug targets and already being developed for their therapeutic potential.

Cross-References

- ▶ CTLA-4
- ▶ CTLA4-Ig
- ▶ Cytotoxic T Lymphocytes
- ▶ Genetics of Juvenile Idiopathic Arthritis
- ▶ Juvenile Idiopathic Arthritis: Pathogenesis, Presentation, and Treatment
- ▶ PI3K
- ▶ Rheumatoid Arthritis, Treatment
- ▶ SH2 Domain-containing Inositol Phosphatase-1 (SHIP)
- ▶ T Cell Memory
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver
- ▶ Tumor-Infiltrating T Cells

References

Barach YS, Lee JS, Zang X. T cell coinhibition in prostate cancer: new immune evasion pathways and emerging therapeutics. Trends in Molecular Medicine. 2011;17:47–55.

Bour-Jordan H, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M, Bluestone JA. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. Immunological Reviews. 2011;241:180–205.

Chattopadhyay K, Lazar-Molnar E, Yan Q, Rubinstein R, Zhan C, Vigdorovich V, et al. Sequence, structure, function, immunity: structural genomics of costimulation. Immunological Reviews. 2009;229:356–86.

Collins M, Ling V, Carreno BM. The B7 family of immune-regulatory ligands. Genome Biology. 2005;6:223.

Crotty S. Follicular helper CD4 T cells (TFH). Annual Review of Immunology. 2011;29:621–63.

Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. Nature Immunology. 2002;3:611–8.

Felix NJ, Suri A, Salter-Cid L, Nadler SG, Gujrathi S, Corbo M, et al. Targeting lymphocyte co-stimulation: from bench to bedside. Autoimmunity. 2010;43:514–25.

Hofmeyer KA, Ray A, Zang X. The contrasting role of B7-H3. Proceedings of the National Academy of Sciences of the United States of America. 2008;105:10277–8.

Hofmeyer KA, Jeon H, Zang X. The PD-1/PD-L1 (B7-H1) pathway in chronic infection-induced cytotoxic T lymphocyte exhaustion. Journal of Biomedicine & Biotechnology. 2011;2011:451694.

Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annual Review of Immunology. 2008;26:677–704.

Nurieva RI, Liu X, Dong C. Molecular mechanisms of T-cell tolerance. Immunological Reviews. 2011;241:133–44.

Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. International Immunology. 2007;19:813–24.
Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. Immunological Reviews. 2009;229:12–26.
Scandiuzzi L, Ghosh K, Zang X. T cell costimulation and coinhibition: genetics and disease. Discovery Medicine. 2011;12:119–28.
Schwartz JC, Zhang X, Nathenson SG, Almo SC. Structural mechanisms of costimulation. Nature Immunology. 2002;3:427–34.
Sharma P, Wagner K, Wolchok JD, Allison JP. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. Nature Reviews Cancer. 2011;11:805–12.
Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nature Immunology. 2007;8:239–45.
Yong PF, Salzer U, Grimbacher B. The role of costimulation in antibody deficiencies: ICOS and common variable immunodeficiency. Immunological Reviews. 2009;229:101–13.
Zang X, Allison JP. The B7 family and cancer therapy: costimulation and coinhibition. Clinical Cancer Research. 2007;13:5271–9.
Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nature Reviews Immunology. 2008;8:467–77.

BAFF and APRIL and Their Receptors

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Synonyms

APRIL (a proliferation ligand): TALL-2, TNFSF13; BAFF (B cell activating factor): zTNF4, BLyS, TALL-1, THANK, TNFSF13B; BAFF-R: Bcmd1, BR3, TNFRSF13C, CD268; BCMA: TNFRSF13A, CD269; TACI: TNFRSF13B, CD267

Definition

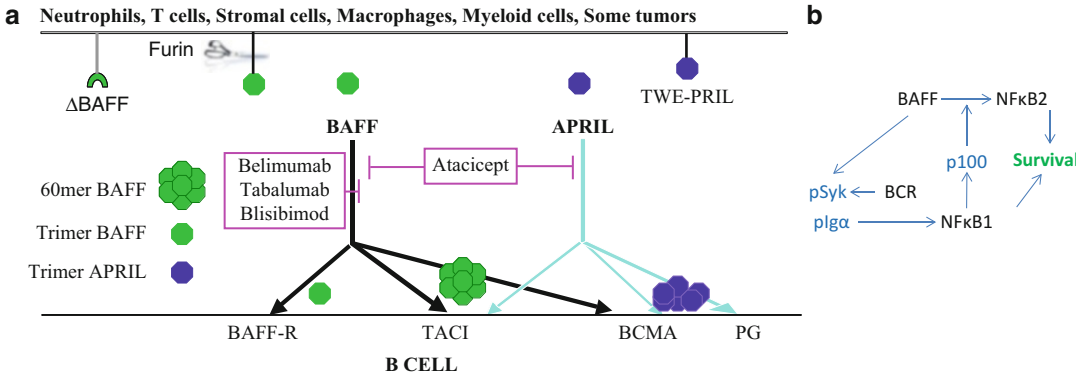
BAFF and APRIL are homologous homotrimeric cytokines belonging to the TNF family.

BAFF and APRIL Ligands and Receptors

BAFF (B cell activating factor: also named zTNF4, BLyS, TALL-1, THANK, TNFSF13B) and APRIL (a proliferation ligand: also named TALL-2, TNFSF13) are homologous TNF-like cytokines that are made by a wide variety of cell types including myeloid cells, stromal cells, activated lymphocytes, epithelial cells, and some cancer cells. Their major function is to support the survival and differentiation of B cells. BAFF binds to three receptors, BAFF-R (also named Bcmd1, BR3, TNFRSF13C, CD268), TACI (TNFRSF13B, CD267), and BCMA (TNFRSF13A, CD269), that are expressed on B cells at different developmental stages, whereas APRIL binds only to TACI and BCMA (Fig. 1a). BAFF-R is the predominant receptor on transitional, naïve, and memory B cells: TACI, the predominant receptor on marginal zone B cells and short-lived plasma cells, and BCMA, the predominant receptor on long-lived plasma cells (reviewed by Davidson (2010); Mackay et al. (2010); Mackay and Schneider (2009)). BAFF receptors may also be expressed on other immune cell types including activated T cells and dendritic cells (DCs) as well as on non-immune cells such as cancer cells (Vincent et al. 2013).

BAFF and APRIL are type II transmembrane proteins that are cleaved either at the cell membrane (BAFF) or in the Golgi apparatus (APRIL) to generate soluble forms. Several splice isoforms of both cytokines exist including ΔBAFF, a transmembrane form lacking an exon that may act as a negative regulator of BAFF function, and TWE-PRIL, a fusion protein of the extracellular domain of APRIL with the intracellular domain of TWEAK, thereby yielding a transmembrane form of APRIL. BAFF and APRIL may also form a small amount of heterodimers (reviewed by Mackay and Schneider (2009); Vincent et al. (2013)).

In mice, BAFF-R is a high affinity receptor for BAFF and binds to BAFF in its trimeric form. Although the BAFF trimer can bind to TACI, activation of the TACI receptor by BAFF requires multimerization to a 60-mer form



BAFF and APRIL and Their Receptors, Fig. 1 (**a**) BAFF, APRIL, and their receptors. BAFF is generated as a cell surface molecule that is cleaved by a furin protease. BAFF circulates either as a trimer but also forms 60 mers made of 20 trimers that have a higher affinity than the trimer and can activate TACI. APRIL is cleaved intracellularly and secreted as a trimer that can be multimerized by binding to proteoglycans on cell surfaces. BAFF binds to all three receptors with varying affinities (BAFF-R > TACI > BCMA). APRIL binds to TACI and BCMA. Alternate forms of the ligands and receptors include ΔBAFF, a cell surface form missing an exon and TWE-PRIL, a fusion of APRIL with the intracellular domain of TWEAK. The interaction of the ligands and receptors can be blocked by antibodies to BAFF (belimumab and tabalumab) or fusion proteins of the receptors with Fc (atacicept, blisibimod). These may interfere with the binding of BAFF to its three receptors, leaving APRIL functions intact BAFF (belimumab, tabalumab, and blisibimod) or may interfere with the binding of both BAFF and APRIL with all three receptors (atacicept). (**b**) Signaling interactions between BAFF-R and BCR. In late transitional cells, BCR signals through the NFkB1 pathway provide p100, a substrate for the NFkB pathway activated by BAFF-R. Conversely, BAFF-R enhances BCR-mediated signals by increasing phosphorylation of Syk and Igα. Together these signals cooperate to enhance B cell survival. If the BCR is downregulated as a result of encounter with autoantigen in the early transitional phase, there will be an increased dependence on BAFF

(Bossen et al. 2008). APRIL similarly binds better to its receptors when it is multimerized, in this case by binding to heparin sulfate proteoglycans on cell surfaces (Ingold et al. 2005) (Fig. 1a).

The Role of BAFF/APRIL in Naïve B Cell Development and Survival

Neither BAFF nor APRIL is required for the maturation of B cells in the bone marrow; however, the interaction of BAFF with BAFF-R is essential for the survival of B cells past the early transitional (T1) stage with only a minor contribution from TACI and none from APRIL or BCMA (reviewed by Davidson (2010); Mackay and Schneider (2009)). The earliest transitional cells are subject to deletion or anergy induction when they receive a BCR signal, because their immature rafts contain insufficient cholesterol to assemble signaling molecules. In the transitional type 2 (T2) stage, BCR signaling through the classical NFkB pathway upregulates expression of BAFF-R and also generates p100, an essential substrate for the non-classical NF-κB signaling pathway used by BAFF-R (Stadanlick et al. 2008). Upon receiving both BCR- and BAFF-mediated signals, T2 cells can now differentiate and migrate either to the marginal zone or to the B cell follicles, where they require a source of BAFF for their continued survival. BAFF-R signaling also enhances phosphorylation of Syk, a signaling molecule required for optimal BCR signaling (Schweighoffer et al. 2013) (Fig. 1b). This cross-talk between the BCR and BAFF-R is essential for the survival of most transitional type 2, marginal zone and mature naive B cells.

Autoreactive B cells are particularly dependent on BAFF, because if they downregulate their BCR as a consequence of antigen encounter

at the T1 stage, they will produce less p100 and compete poorly for BAFF as they progress to the T2 stage. When B cell numbers and BAFF levels are normal, stringent deletion of autoreactive B cells occurs. However, an increase in serum BAFF levels, such as occurs during B cell lymphopenia or perhaps during inflammatory states, may result in relaxation of B cell selection, with survival of more autoreactive B cells (reviewed by Liu 2011). Importantly however, the effect of excess BAFF on naïve B cell selection can be quite variable, and not all autoreactive B cells are equally susceptible to either excess BAFF or BAFF inhibition at the transitional B cell checkpoint (reviewed by Liu 2011). Furthermore, excess BAFF not rescue autoreactive B cells that are targeted for deletion in the bone marrow.

In addition to cross-talk with the BCR, TACI and BAFF-R also interact with the TLR system. Stimulation through either TACI or BAFF-R results in upregulation of endosomal TLRs Katsenelson et al. (2007); TACI also binds to the TLR adaptor MyD88, although at a different site than do the TLR molecules (He et al. 2010). Conversely, TLR signals can upregulate expression of both TACI and BAFF-R on B cells, rendering them more sensitive to BAFF or APRIL signals (Treml et al. 2007). This innate immune amplification loop may enhance inflammation in the setting of chronic diseases.

TACI mutations are found in approximately 10 % of CVID patients. The causative mechanism is complex since there is incomplete penetrance of an immune deficient phenotype in family members with the same TACI mutations (Salzer et al. 2009). BAFF-R mutations are more rarely associated with CVID, and similar to TACI, there is variable penetrance within families.

The Role of BAFF/APRIL in Antigen-Driven B Cell Development and Survival

The role of BAFF and APRIL and their receptors changes once B cells begin to encounter antigen. The most important receptor for T cell–independent humoral responses is TACI that is highly expressed on B1 and marginal zone B cells and on the short-lived plasma cells that arise during the T-independent response (Mackay and Schneider 2008). Both BAFF and APRIL can mediate these responses, but the interaction of TACI with APRIL is relatively more important than that of TACI with BAFF for IgA responses and IgA is deficient in APRIL knockout mice (reviewed by Mackay and Schneider 2009). By contrast, BAFF and BAFF-R are required for optimal germinal center responses and germinal centers are smaller and of shorter duration when either the ligand or receptor is missing (reviewed by Liu and Davidson 2011b; Kalled 2006). Whether BAFF regulates selection of B cells in the germinal center is still not known.

Although BAFF receptors are expressed on memory B cells, maintenance of class switched B cell memory appears to be independent of either BAFF or APRIL in both mice and humans (Scholz et al. 2008; Benson et al. 2008; Jacobi et al. 2010), although there is some in vitro data suggesting that BAFF may costimulate memory B cell reactivation in the setting of inflammation. In adult mice, the establishment of long-lived plasma cells in the bone marrow following immunization is dependent on both BAFF and APRIL via their interaction with BCMA and TACI. Nevertheless, other components of the bone marrow niche may substitute for BAFF and APRIL once the niche is established or during inflammation (Ramanujam and Davidson 2008). Indeed, in humans, there is only a modest effect of BAFF/APRIL inhibition on antibody titers to recall antigens such as tetanus toxin and influenza. Mouse BAFF binds only weakly to the BCMA receptor, and since expression of TACI in neonatal mice is poor, neonatal plasma cells depend on the interaction between APRIL and BCMA and are deficient in APRIL knockout mice. Since human BAFF binds to BCMA, it is not clear whether this finding also pertains to humans, but B cells from infants express less BAFF receptors than do adult B cells.

The Role of BAFF and APRIL in Other Cell Types

BAFF-R is expressed on activated T cells and the interaction of BAFF with BAFF-R on CD4 T cells enhances their production of IFNγ and IL-17 (Zhou et al. 2011). Modulation of BAFF-R on T cells has been shown to influence the severity of transplant rejection and the progression of the autoimmune disease, Systemic Lupus Erythematosus (SLE). BAFF also supports the survival of monocytes and enhances their differentiation. Human myeloid DCs stimulated in vitro with BAFF upregulate co-stimulatory molecules, lose phagocytic ability, and produce inflammatory cytokines (Chang et al. 2008). In a mouse model of arthritis, BAFF silencing in synovial DCs prevented their maturation; these cells failed to produce the IL-6 required for the differentiation of T helper type 17 (Th17) cells (Lai Kwan Lam et al. 2008); IL-6 is also required for optimal plasma cell differentiation and for the differentiation of regulatory T cells.

BAFF and APRIL and their receptors are upregulated during infections as a result of a response to Type I interferons and increased TLR expression. Inappropriate BAFF and APRIL secretion in the setting of chronic infections has been linked to a potential risk for hematologic malignancy. BAFF and APRIL have also been implicated in the maintenance of some malignancies including CLL and multiple myeloma (reviewed by Vincent et al. 2013).

Signaling Through BAFF Receptors

Signaling through BAFF-R activates the alternative NFκB pathway by depleting the TRAF3 protein that constitutively suppresses activation of NF-κB2. BAFF enhances long-term B cell survival primarily through this pathway by upregulating anti-apoptotic proteins including Mcl-1. Signaling through BAFF-R also activates several other important survival pathways. Signaling through Mek/Erk (extracellular signal regulated kinase) and through Akt/mTOR (molecular target of rapamycin) mediate cell survival by repressing the proapoptotic protein Bim (reviewed in Mackay et al. 2010). BAFF-R signaling prevents nuclear translocation of protein kinase Cδ (PKCδ), preventing its proapoptotic effects. BAFF-R ligation results in signals through Pim2 that increase metabolic fitness by inducing a metabolic bias toward glycolysis. In this way, BAFF keeps B cells in a state of readiness to respond to BCR activation. Finally, as mentioned above, signals through BAFF-R induce phosphorylation of both Syk and the BCR-associated Igα signaling subunit, and therefore amplify BCR-mediated signals (Schweighoffer et al. 2013).

TACI is a potent stimulator of the classical NF-κB1 pathway and also activates both the Jnk (c-Jun N-terminal kinase)/p38 pathway and calcium-modulating cyclophilin ligand (CAML), which leads to activation of nuclear factor for activated T cells (NFAT) (reviewed in Mackay et al. (2010)). As mentioned above, TACI also interacts directly with MyD88 and amplifies innate immune signals through this mechanism (He et al. 2010). TACI has also been considered a negative signaling molecule because TACI deficiency results in B cell expansion and activation (Mackay and Schneider 2008). However, this may be due to a relative increase in BAFF and APRIL availability when TACI is absent, resulting in enhanced binding of BAFF to BAFF-R.

BCMA is a target of the plasma cell transcription factor BLIMP1 and is expressed on plasma cells as well as on several different types of tumor. Like TACI, BCMA activates the classical NFκB pathway as well as the JNK, p38, and ERK pathways.

BAFF and Autoimmunity

BAFF transgenic mice that have 30–100 fold increased BAFF levels develop a mild form of autoimmunity with features of Sjogren's syndrome and SLE. Conversely, inhibition of BAFF or of both BAFF and APRIL attenuates lupus in

multiple mouse models. The mechanism for the induction of autoimmunity in normal mice by transgenic expression of high concentrations of BAFF is due to T cell–independent but MyD88-dependent activation of innate B cells that produce autoantibodies, particularly those of the IgA isotype. High levels of BAFF also result in loss of stringency of B cell selection and may promote isotype switching in a T cell–independent manner. No autoimmunity is induced in APRIL transgenic mice, but these mice develop B1 cell lymphomas with age (reviewed by Mackay and Schneider 2009).

High levels of BAFF (2–5 folds increased) have been observed in a proportion of patients with multiple autoimmune and inflammatory diseases, and high local expression of BAFF has been observed in inflamed organs. These observations have led to the development of several drugs that inhibit either BAFF alone or both BAFF and APRIL. These drugs include antibodies directed at BAFF or fusion proteins of the BAFF-R or TACI receptors with the Fc portion of Ig. Although BAFF inhibition abrogates arthritis in mouse models, several BAFF antagonists have failed in human clinical trials of rheumatoid arthritis. Disappointingly, the first use of the BAFF/APRIL inhibitor atacicept (TACI-Ig) in multiple sclerosis resulted in disease worsening, by mechanisms that are not well understood. The recent finding that BAFF may bind to the CNS receptor Nogo-66 may help to explain these findings (reviewed by Vincent et al. 2013). By contrast, the first anti-BAFF antibody to be developed, belimumab, was successful in two large Phase 3 studies of SLE and was approved by the FDA for the treatment of SLE in 2011. Studies of large numbers of SLE patients treated with either standard of care therapy or standard of care therapy with belimumab have shown that belimumab induces a 30–40 % decrease in circulating autoantibody titers, an increase in serum complement levels, and a significantly greater clinical response than in patients receiving standard of care therapy alone. Post-hoc analyses have indicated that the drug is most effective in a subset of patients with high levels of serum anti-DNA levels and low complement levels (reviewed by Boneparth and Davidson 2012).

How Does BAFF Inhibition Work?

Two types of BAFF inhibitors have been developed. Selective inhibitors block the interaction of BAFF with BAFF-R whereas the non-selective inhibitor TACI-Ig blocks both BAFF and APRIL, preventing the interaction of both cytokines with all their receptors (Fig. 1a). Both types of inhibitors effectively prevent SLE in mouse models, suggesting that the interaction of BAFF with BAFF-R is the dominant interaction that mediates this disease. Nevertheless, studies using knockout mice have shown that while deficiency of BAFF attenuates disease in lupus models, absence of BAFF-R has no effect. This could be because the profound B cell depletion in BAFF-R deficient mice results in a marked increase in serum BAFF levels, recapitulating the physiology of the BAFF transgenic mouse. As discussed above, TACI-Ig has a greater effect than selective BAFF inhibitors on both short-lived and long-lived plasma cells although the decrease in serum levels of IgM and IgA is considerably greater than the decrease of serum IgG. The mechanism for this difference, whether it is intrinsic to the switched cells or to their environment, is not known.

Mechanistic studies in SLE-prone mice have shown that both BAFF-R-Ig and TACI-Ig deplete B cells past the T1 stage but have only modest effects on IgG autoantibody production and renal immune complex deposition and no apparent effect on somatic mutation or autoantibody affinity. Nevertheless, there is a significant attenuation of renal disease. Whether this is due to an alteration in the pathogenicity of the deposited autoantibodies has not yet been determined. Part of this effect may be secondary to B cell depletion with a decrease in the overall size of secondary lymphoid organs and therefore a decrease in the total number of activated inflammatory cells. An alternate explanation is that there is a direct

inhibition of renal inflammation through effects on renal cells (reviewed by Liu and Davidson 2011b).

Studies in humans with SLE have shown treatment with the anti-BAFF antibody belimumab depletes peripheral blood naïve B cells and non-class switched memory cells but not class switched memory cells. Indeed an unexplained finding in humans is a two fold increase in CD19+/CD27+ cells within 2–4 weeks of starting either belimumab or TACI-Fc (atacicept) therapy. There is a modest effect on circulating plasmablasts with a preferential effect on IgM- and IgA- rather than IgG-producing cells (Jacobi et al. 2010). These results are consistent with observations in the mouse models. More work is needed to determine whether BAFF inhibition alters selection of human B cells or impacts on the function of T cells or DCs.

The Future of BAFF and APRIL Therapeutic Targeting

Based on the initial success of belimumab in the treatment of SLE, a number of other BAFF and BAFF/APRIL inhibitors have been developed (reviewed by Liu and Davidson 2011b; Fairfax et al. 2012). Selective inhibitors include other antibodies to BAFF (Tabalumab) or fusion proteins of BAFF-R or part of BAFF-R with Fc (Blisibimod). The non-selective inhibitor atacicept is a fusion protein of TACI with Fc. Potential indications include other autoimmune diseases in which B cells play an important role and/or BAFF levels are elevated as well as B cell malignancies and transplant rejection. Clinical trials in several of these indications are planned. Atacicept has a more robust effect on plasma cell survival than do the selective inhibitors and in patients with SLE, high doses have been associated with a decrease in serum immunoglobulin levels and an increased rate of infections. Nevertheless, atacicept should be a more efficacious reagent than selective BAFF inhibitors with respect to elimination of plasma cells. The next few years should see results of a large number of clinical trials that should better clarify the indications for BAFF and BAFF/APRIL inhibitors and their mechanism of action.

Cross-References

- ▶ B Cell Tolerance
- ▶ Novel Targets in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Animal Models

References

Benson MJ, Dillon SR, Castigli E, Geha RS, Xu S, Lam KP, et al. Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. J Immunol. 2008;180(6):3655–9.

Boneparth A, Davidson A. B-cell activating factor targeted therapy and lupus. Arthritis Res Ther. 2012;14 Suppl 4:S2.

Bossen C, Cachero TG, Tardivel A, Ingold K, Willen L, Dobles M, et al. TACI, unlike BAFF-R, is solely activated by oligomeric BAFF and APRIL to support survival of activated B cells and plasmablasts. Blood. 2008;111(3):1004–12.

Chang SK, Mihalcik SA, Jelinek DF. B lymphocyte stimulator regulates adaptive immune responses by directly promoting dendritic cell maturation. J Immunol. 2008;180(11):7394–403.

Davidson A. Targeting BAFF in autoimmunity. Curr Opin Immunol. 2010;22(6):732–9.

Fairfax K, Mackay IR, Mackay F. BAFF/BLyS inhibitors: a new prospect for treatment of systemic lupus erythematosus. IUBMB Life. 2012;64(7):595–602.

He B, Santamaria R, Xu W, Cols M, Chen K, Puga I, et al. The transmembrane activator TACI triggers immunoglobulin class switching by activating B cells through the adaptor MyD88. Nat Immunol. 2010;11(9):836–45.

Ingold K, Zumsteg A, Tardivel A, Huard B, Steiner QG, Cachero TG, et al. Identification of proteoglycans as the APRIL-specific binding partners. J Exp Med. 2005;201(9):1375–83.

Jacobi AM, Huang W, Wang T, Freimuth W, Sanz I, Furie R, et al. Effect of long-term belimumab treatment on b cells in systemic lupus erythematosus: extension of a phase II, double-blind, placebo-controlled, dose-ranging study. Arthritis Rheum. 2010;62(1):201–10.

Kalled SL. Impact of the BAFF/BR3 axis on B cell survival, germinal center maintenance and antibody production. Semin Immunol. 2006;18(5):290–6.

Katsenelson N, Kanswal S, Puig M, Mostowski H, Verthelyi D, Akkoyunlu M. Synthetic CpG oligodeoxynucleotides augment BAFF- and APRIL-mediated immunoglobulin secretion. Eur J Immunol. 2007;37(7):1785–95.
Lai Kwan Lam Q, King Hung Ko O, Zheng BJ, Lu L. Local BAFF gene silencing suppresses Th17-cell generation and ameliorates autoimmune arthritis. Proc Natl Acad Sci USA. 2008;105(39):14993–8.
Liu Z, Davidson A. BAFF and selection of autoreactive B cells. Trends Immunol. 2011a;32(8):388–94.
Liu Z, Davidson A. BAFF inhibition: a new class of drugs for the treatment of autoimmunity. Exp Cell Res. 2011b;317(9):1270–7.
Mackay F, Schneider P. TACI, an enigmatic BAFF/APRIL receptor, with new unappreciated biochemical and biological properties. Cytokine Growth Factor Rev. 2008;19(3–4):263–76.
Mackay F, Schneider P. Cracking the BAFF code. Nat Rev Immunol. 2009;9(7):491–502.
Mackay F, Figgett WA, Saulep D, Lepage M, Hibbs ML. B-cell stage and context-dependent requirements for survival signals from BAFF and the B-cell receptor. Immunol Rev. 2010;237(1):205–25.
Ramanujam M, Davidson A. BAFF blockade for systemic lupus erythematosus: will the promise be fulfilled? Immunol Rev. 2008;223:156–74.
Salzer U, Bacchelli C, Buckridge S, Pan-Hammarstrom Q, Jennings S, Lougaris V, et al. Relevance of biallelic versus monoallelic TNFRSF13B mutations in distinguishing disease-causing from risk-increasing TNFRSF13B variants in antibody deficiency syndromes. Blood. 2009;113(9):1967–76.
Scholz JL, Crowley JE, Tomayko MM, Steinel N, O'Neill PJ, Quinn 3rd WJ, et al. BLyS inhibition eliminates primary B cells but leaves natural and acquired humoral immunity intact. Proc Natl Acad Sci USA. 2008;105(40):15517–22.
Schweighoffer E, Vanes L, Nys J, Cantrell D, McCleary S, Smithers N, et al. The BAFF receptor transduces survival signals by co-opting the B cell receptor signaling pathway. Immunity. 2013;38(3):475–88.
Stadanlick JE, Kaileh M, Karnell FG, Scholz JL, Miller JP, Quinn 3rd WJ, et al. Tonic B cell antigen receptor signals supply an NF-kappaB substrate for prosurvival BLyS signaling. Nat Immunol. 2008;9(12):1379–87.
Treml LS, Carlesso G, Hoek KL, Stadanlick JE, Kambayashi T, Bram RJ, et al. TLR stimulation modifies BLyS receptor expression in follicular and marginal zone B cells. J Immunol. 2007;178(12):7531–9.
Vincent FB, Saulep-Easton D, Figgett WA, Fairfax KA, Mackay F. The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. Cytokine Growth Factor Rev. 2013;24(3):203–15.
Zhou X, Xia Z, Lan Q, Wang J, Su W, Han YP, et al. BAFF promotes Th17 cells and aggravates experimental autoimmune encephalomyelitis. PLoS One. 2011;6(8):e23629.

Bcl-2 Family Members and Lymphocyte Homeostasis

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Synonyms

A1, Bcl-2-related protein A1, BCL2L5, BFL1; Bad, BCL2-associated agonist of cell death, BBC2, BCL2L8; Bak, BCL2-antagonist/killer, BCL2L7; Bax, BCL2-associated X protein, BCL2L4; Bcl-2, B cell lymphoma 2, PPP1R50; Bcl-xL, BCL2L1, Bcl-X, PPP1R52; Bid, BH3 interacting domain death agonist; Bik, BCL2-interacting killer; Bim, BCL2L11; Bok, BCL2-related ovarian killer, BCL2L9, BOKL; Hrk, harakiri Bcl-2 interacting protein; Mcl-1, myeloid cell leukemia sequence 1, BCL2L3; Noxa; Puma

Definition

Regulating T and B cell development and peripheral homeostasis is critical to prevent aberrant immune responses. Apoptosis is a form of programmed cell death crucial for maintaining homeostasis of T and B cells. During T and B cell development, apoptosis is involved in eliminating self-reactive cells, which is necessary to prevent autoimmunity. In the case of an infection, activated T and B cells undergo expansion in response to antigen stimulation and subsequently develop effector functions. After this expansion period, most cells are eliminated through apoptosis and some develop into memory cells.

This selective process of contraction and memory development is crucial in resetting T and B cell homeostasis and promoting protective immunity. Studies show that the Bcl-2 family members are critical regulators of apoptosis, contributing to the regulation of T and B cell development and contraction of immune responses. The focus of this review will be on the Bcl-2 family members that are critical in determining T and B cell fate during development, homeostasis and activation, and how functional mutations in the Bcl-2 family members may lead to autoimmunity (▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis).

Introduction

The Apoptotic Pathways

There are two major pathways of apoptosis – the death receptor-mediated or extrinsic pathway and the Bcl-2-regulated or intrinsic pathway. The extrinsic apoptotic pathway (▶ Fas/Fas Ligand) is initiated by the trimerization of a target cell's death receptors to their cognate death ligands. The death ligands are a part of the tumor necrosis factor (TNF) family and include TNF-α, Fas ligand (FasL/CD95L), and TNF-related apoptosis-inducing ligand (TRAIL). The death receptors, such as Fas/CD95 and TRAIL-receptor 1, contain death domains that recruit pro-caspase 8 through the protein Fas-associated death domain (FADD), resulting in caspase-8 activation and subsequent activation of the effector caspases 3, 6, and 7 (Kurtulus et al. 2010). While death receptor signaling plays an essential role in lymphocyte homeostasis, its importance has been discussed elsewhere and will not be a major subject of this review.

The intrinsic mitochondrial pathway is initiated by a variety of signals, such as cytokine/growth factor withdrawal, T/B cell receptor (TCR/BCR) crosslinking, and DNA damage (Strasser 2005). These signals alter the expression and activation state of anti- and pro-apoptotic Bcl-2 family members, thereby controlling cell survival. The Bcl-2 family is comprised of anti-apoptotic and pro-apoptotic proteins that have up to four α-helix Bcl-2 homology domains (Youle and Strasser 2008). The anti-apoptotic proteins Bcl-2, Bcl-xL, and Bcl-W contain BH1-4, A1 contains BH1-2, and Mcl-1 contains BH1-3 domains. Genetically deficient mice have revealed roles for Bcl-2 and Mcl-1, but not Bcl-xL, in hematopoietic and lymphocyte survival; roles for Bcl-xL in neuronal and embryonic development and thymocytes, but not peripheral T cells; and roles for Mcl-1 and Bcl-xL in reproductive organ development (Youle and Strasser 2008). These anti-apoptotic molecules are crucial for protecting cells from death driven by pro-apoptotic molecules. Pro-apoptotic Bcl-2 family members contain two major subgroups: (i) the Bax-/Bak-like molecules including Bax, Bak, and Bok which contain BH1-3 domains and (ii) a second larger class of pro-apoptotic proteins (Bim, Bad, Bid, Bmf, Noxa, Puma, Nik, Bik, Hrk) whose only homology to Bcl-2 is a 9–10 amino acid region called the BH3 domain. This BH3 domain is essential for the ability of BH3-only molecules to drive apoptosis. Generation of genetically deficient animals has revealed roles for Bax in sperm cell development; Bim in thymocyte negative selection and peripheral T cell homeostasis; Puma and Noxa in responses to DNA damage stimuli; and Bad, Bim, and Puma in growth factor withdrawal of lymphocytes and other cell types (Youle and Strasser 2008). Although most cell types express multiple Bcl-2 family members, there is a degree of tissue-specific expression, particularly in terms of expression of BH3-only molecules. In addition, recent data suggest there is specificity and hierarchy that governs mechanistic interactions among Bcl-2 family members.

An important hierarchical relationship between pro-apoptotic Bcl-2 family members was uncovered when it was found that the ability of BH3-only proteins to induce apoptosis required the expression of both Bax and Bak (Lindsten et al. 2000; Zong et al. 2001). There are two proposed models that explain Bax-/Bak-like molecule activation (Kurtulus et al. 2010). The first model proposes that BH3-only proteins sequester anti-apoptotic proteins away

from Bax-/Bak-like molecules, inducing their self-oligomerization and activation. The second model proposes that BH3-only proteins interact directly with Bax-/Bak-like molecules and thereby induce their activation. Evidence exists supporting both models, and it is important to note that these models likely are not mutually exclusive, as the cell type and dominantly expressed Bcl-2 family members may govern how apoptosis is initiated. Indeed, one critical study genetically swapped the BH3 domain of pro-apoptotic molecules in knockin mice and demonstrated that both mechanisms are likely involved in initiating apoptosis (Merino et al. 2009). It is also possible that both mechanisms are operative simultaneously in the same cell. Whatever the mechanism, the activation of Bax-/Bak-like molecules drives mitochondrial outer membrane permeabilization and the release of cytochrome c. Cytosolic cytochrome c forms a complex with pro-caspase 9 and apoptotic protease-activating factor-1 (APAF-1) called the apoptosome, leading to caspase 9 activation and subsequent effector caspase activation (Opferman 2008). Both the extrinsic and intrinsic pathways lead to florid caspase activation, which is critical for the morphologic features associated with apoptosis.

Bcl-2 Family Members in T Cell Development, Homeostasis, and Activation

T Cell Development

T cell development in the thymus goes through several checkpoints to ensure that a functional T cell receptor (TCR) is rearranged and that the TCR does not recognize self-antigens with enough avidity to drive overt autoimmunity. Bcl-2 family members play critical roles to ensure only thymocytes that produced a functional TCR with adequate avidity to self-antigen/major histocompatibility complex (MHC) complexes survive. At earlier stages (up to the double-positive (DP) stage), thymocytes are dependent on IL-7 signaling (Peschon et al. 1994). IL-7 may function through multiple mechanisms to promote thymocyte survival as it has been shown to induce expression of Bcl-2 (Kim et al. 1998) and Mcl-1 (Opferman et al. 2003), as well as to inhibit the expression of Bax (Khaled et al. 1999; Kim et al. 1998) and Bad (Li et al. 2004). In mice, transgenic overexpression of Bcl-2 or the loss of Bim can restore thymocyte atrophy in IL-7Rα-deficient mice, suggesting the intersection of IL-7 signaling with prevention of apoptosis in vivo (Akashi et al. 1997; Pellegrini et al. 2004). How IL-7 normally controls pro- versus anti-apoptotic Bcl-2 family member expression/function remains incompletely understood, although some mechanistic details are emerging. For example, it was shown that the loss of Bax could restore early thymocyte development in the absence of IL-7Rα or in mice deficient in Bcl-2 (Khaled et al. 2002). However, in neither of these instances was the absence of Bax able to restore peripheral T cells. Thus, it is likely that Bax plays stage-specific roles in controlling Bim-mediated apoptosis. Indeed, mice deficient in Bax and Bim have a distinct, albeit subtle, thymic phenotype compared to mice deficient in Bak and Bim (Hutcheson et al. 2005). Thus, early in thymocyte development (or at an earlier stage), IL-7Rα/Bcl-2 may counteract Bax and Bim, but at later stages, and in peripheral T cells, Bim may also signal through Bak to promote T cell death. Why there is differential involvement of Bax and Bak at these early stages is unclear, but is likely reflective of Bak function rather than simple lack of Bak expression.

The anti-apoptotic molecule Mcl-1 may be an important clue here, as Mcl-1 can be induced by IL-7 and it regulates the survival of thymocytes at earlier stages (Opferman et al. 2003) by inhibiting Bak-mediated death in a way that is independent of Bcl-2, Bax, and Bim (Dunkle et al. 2010). In support of this model, another study showed that Mcl-1 deficiency-induced neutropenia could be rescued by deletion of Bax and Bak, but not Bim or PUMA (Steimer et al. 2009). These studies suggest a model where Bim and Bcl-2 balance-regulated survival is upstream of Mcl-1 and Mcl-1 inhibits Bak- mediated death in thymocytes.

As thymocytes begin to rearrange TCR-β chain, they go through a process called "β-selection" which requires ligand-independent signaling through a functional pre-TCR for survival and differentiation of thymocytes. Bcl-2 does not protect thymocytes from apoptosis at this stage (Maraskovsky et al. 1997). One other Bcl-2 family protein, A1, has been shown to be induced by pre-TCR signaling (Mandal et al. 2005). Further, knockdown of A1 can decrease survival of pre-T cell lines, whereas its overexpression in $Rag1^{-/-}$ thymocytes can induce development into DP stage (Mandal et al. 2005). Unfortunately, these results could not be confirmed by genetic deletion of the A1d gene in mice, which is abundantly expressed in pre-T cells.

As the cells start to rearrange TCR α chain, they are tested for successful TCR rearrangement. T cells that do not express a functional TCR undergo death by neglect; the ones with high affinity for self-peptide/MHC complexes die by negative selection, and DP thymocytes that are positively selected by proper interactions between TCR and peptide/MHC complexes transit into $CD4^+$ or $CD8^+$ single-positive (Lenschow et al. 1994) thymocytes. During this selection process, survival of DP thymocytes is provided by Bcl-xL (Grillot et al. 1995; Ma et al. 1995; Motoyama et al. 1995; Zhang and He 2005). Overexpression of Bcl-xL protects DP thymocytes from a variety of apoptotic stimuli including anti-CD3 stimulation (Grillot et al. 1995). However, Bcl-xL overexpression does not protect DP thymocytes from negative selection (Chao and Korsmeyer 1997; Grillot et al. 1995). Bcl-2 overexpression, on the other hand, partially rescued DP thymocytes from negative selection as demonstrated in anti-HY TCR transgenic male mice and superantigen-induced deletion models (Strasser et al. 1991a, b). However, Bcl-2 does not restore development of DP thymocytes that fail to be positively selected (Strasser et al. 1994b). This demonstrates the requirement for TCR signals for differentiation and maturation of thymocytes in addition to their survival. While survival is critical for thymocytes at certain stages, simple restoration of survival may not be sufficient to overcome a lack of TCR signals critical for differentiation and maturation of thymocytes.

Negative selection of self-reactive DP thymocytes is critical to prevent autoimmunity. Bim is the critical regulator of the death induced by negative selection. $Bim^{-/-}$ mice have accumulation of lymphocytes in the periphery, and these mice develop spontaneous lymphoid hyperplasia and autoimmunity as they age (Bouillet et al. 1999). Bim deficiency protects DP thymocytes from cell death induced by anti-CD3, Staphylococcus enterotoxin B (SEB) superantigen, or endogenous mouse mammary tumor viral (MMTV) superantigens in vivo (Bouillet et al. 2002). However, the latter result is somewhat controversial as another group found only a partial role for Bim in the negative selection of MMTV-reactive thymocytes (Jorgensen et al. 2007). A possible explanation for this discrepancy may have to do with analysis of the data. For example, thymocytes undergoing negative selection in the absence of Bim downregulate their CD4 and CD8 co-receptors. In most studies these so-called double-negative (DN) cells are excluded from analysis, as most people gate on DP thymocytes to analyze the population of MMTV-reactive cells. However, the majority of MMTV-reactive cells in mice that lack Bim reside in the DN compartment. Similar results in terms of thymocyte negative selection as well as accumulation of aberrant DN cells are observed in mice lacking expression of Bax and Bak. Thus, it appears that, during negative selection, Bim is activated and drives apoptosis of autoreactive thymocytes by activating Bax and Bak. Mechanism(s) that underlie Bim activation are unclear. One component likely involves decreased expression of IL-7Rα along with the Bim antagonist Bcl-2, but another mechanism may involve increased expression of Bim (Liston et al. 2004).

Peripheral T Cell Homeostasis

When single-positive (SP) T cells egress from thymus into the peripheral organs, they are maintained by common γ chain cytokines, mainly IL-7, and TCR-self-peptide/MHC

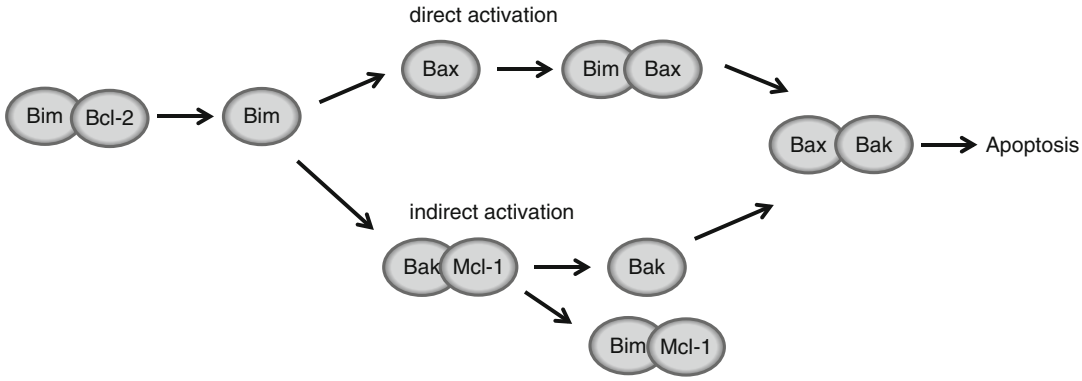
interactions (Surh and Sprent 2008). The downstream targets of TCR signaling that are important for naïve T cell homeostasis remain unclear. However, IL-7 signaling can enhance expression of the anti-apoptotic proteins Bcl-2 and Mcl-1 (Kondrack et al. 2003; Opferman et al. 2003), which are highly expressed in naïve $CD4^+$ and $CD8^+$ T cells (Hildeman et al. 2007). In vitro, IL-7 was unable to fully rescue naïve T cells from apoptosis in the absence of Bcl-2, suggesting that Bcl-2 is downstream of IL-7 signaling and is required for optimal naïve T cell survival (Wojciechowski et al. 2007). In support of this in vitro study, in vivo overexpression of Bcl-2 restores naïve T cell homeostasis in the absence of IL-7R signaling (Akashi et al. 1997; Maraskovsky et al. 1997).

Despite the complex biochemical interactions, genetic models have shown that Bcl-2 is critical for naïve T cell survival through combating Bim. This has been demonstrated using $Bim^{+/-}$ $Bcl2^{-/-}$ mice, which have a dramatic loss in total number of naïve $CD8^+$ T cells, loss that is restored with the deletion of the second Bim allele ($Bim^{-/-}$ $Bcl2^{-/-}$ mice) (Bouillet et al. 2001; Wojciechowski et al. 2007). Interestingly, naïve $CD4^+$ T cells were not as sensitive to the absence of Bcl-2, suggesting an additional anti-apoptotic protein (possibly Mcl-1) is critical in antagonizing Bim in naïve $CD4^+$ cells (Wojciechowski et al. 2007). Indeed, in naïve T cells, Bim has been found complexed to Bcl-2, Bcl-xL, and Mcl-1 (Liu et al. 2006; Opferman et al. 2003). However, deletion of Bcl-xL did not affect naïve T cell survival, although it is unclear if Bcl-xL can function redundantly with Bcl-2 in promoting naïve $CD4^+$ T cell survival. Mcl-1 may also be critical in maintaining naïve T cells, as Mcl-1-deficient mice have reduced peripheral $CD4^+$ and $CD8^+$ T cell numbers (Opferman et al. 2003). A recent paper showed that additional loss of Bak, but not Bim, was sufficient to restore naïve T cell survival in Mcl-1-deficient cells in vitro (Dunkle et al. 2010). Thus, these combined data suggest that Mcl-1 functions to antagonize pro-apoptotic molecules downstream of Bim, while Bcl-2 functions largely to antagonize Bim to maintain peripheral $CD8^+$, and to a lesser extent $CD4^+$, T cell survival.

Apoptosis of Activated T Cells

In an acute infection, T cells that are activated undergo 15–20 rounds of division. When the pathogen is cleared, a massive elimination of the activated T cells occurs through apoptotic cell death (Kawabe and Ochi 1991). Early reports suggested that death receptor signaling is required for this clearance of activated T cells (Brunner et al. 1995; Dhein et al. 1995). It is important to note that all of these models required repetitive TCR stimulation, something that is mimicked by chronic infections/autoimmunity, but not during acute responses. Consistent with this, Fas signaling has been reported to be involved in controlling T cell contraction during chronic infection (Hughes et al. 2008; Hutcheson et al. 2008). However, it remains unclear whether the effects of Fas in these models are T cell intrinsic as recent work suggested that the failure of killing germinal center B cells via Fas could contribute to activated T cell accrual (Hao et al. 2008).

Instead, recent data suggest a dominant role for Bcl-2 family members in the contraction of T cell responses to acute infection (Bouillet et al. 1999; Erlacher et al. 2006; Hildeman et al. 2002). Bim appears to be the major molecule required for the death of activated T cells after acute viral infection. The absence of Bim spares more than 80 % of the activated, effector T cells that die in wild-type mice (Hildeman et al. 2002; Wojciechowski et al. 2006). Similar results have been obtained in mice deficient in both Bax and Bak, suggesting that nearly all of the effects of Bim in this model require Bax and Bak (Rathmell et al. 2002). Although overexpression of Bcl-2 or Bcl-xL was initially reported to not impair contraction in viral infection models (Petschner et al. 1998), expression of the transgenes used in these studies was not robust, particularly when cells were activated. Instead, Bcl-2 was found to be critical for the survival of particular subsets of effector $CD8^+$ T cells (▶ Cytotoxic T Lymphocytes), and it protected them from Bim-mediated death



Bcl-2 Family Members and Lymphocyte Homeostasis, Fig. 1 *Models of Bax/Bak activation*. In T cells, the majority of Bim is found complexed to Bcl-2. Following activation, Bcl-2 is transcriptionally downregulated, freeing Bim to either directly interact with Bax and/or Bak (direct activation model) or titrate pro-apoptotic molecules away from Bax/Bak (indirect activation model). Once activated Bax/Bak oligomerize on the mitochondrial outer membrane and drive outer membrane permeability and apoptosis

(Kurtulus et al. 2011) similar to naïve T cells (Wojciechowski et al. 2007). Interestingly, higher expression of Bcl-2 allowed these effector cells to tolerate higher levels of Bim and survive. Effector cells that had lower levels of Bcl-2 were more susceptible to Bim-driven death and were lost during the contraction of the response (Kurtulus et al. 2011). While Bcl-2 appears to be a fulcrum for setting the sensitivity of effector T cells, experiments examining Bim expression in Bax-/Bak-deficient T cells revealed a massive transcriptional upregulation of Bim. Thus, decreased expression of Bcl-2 coupled with transcriptional increases in Bim likely ensures the demise of most activated, effector T cells.

Besides Bim, a role for Puma in activation-induced cell death has been shown in an HSV infection model; although the effect of PUMA deletion was only marginal (Fischer et al. 2008). T cells from $Bim^{-/-}$ $Puma^{-/-}$ mice were more resistant to cytokine withdrawal compared to those from $Bim^{-/-}$ or $Puma^{-/-}$ mice (Erlacher et al. 2006). However, in vivo, the additional loss of Puma did not significantly increase the survival of Bim-deficient T cells.

In addition to Bcl-2, Mcl-1 is also important for the survival of activated T cells in vitro (Dzhagalov et al. 2008). However, the degree to which Mcl-1 combats Bim to maintain effector T cell survival remains unclear. Similar to thymocyte and neutrophil survival (Steimer et al. 2009), Mcl-1 seems to regulate effector T cell survival by inhibiting Bax and/or Bak independent of Bcl-2 and Bim (Tripathi, unpublished data). The anti-apoptotic molecule A1 is also induced in activated T cells (Hildeman et al. 2007; Tomayko et al. 1999). However, its exact role in activated T cell survival in vivo remains to be determined. Thus, current knowledge suggests a model where T cell activation disrupts the balance between Bim and Bcl-2 and unleashed Bim either directly or indirectly activates Bax/Bak molecules by sequestering away Mcl-1 from Bak (Fig. 1). Activated T cells that can navigate a balance between Bim and Bcl-2 can progress into memory cells.

Memory T Cells

Surviving effector T cells go on to become memory T cells (▶ T Cell Memory); a recent study showed that cells expressing low levels of KLRG-1 but high levels of CD127 are enriched for cells with the potential to differentiate into memory T cells. The transitional survival of these effector T cells required both IL-7 and IL-15 (Rubinstein et al. 2008; Tripathi et al. 2010). IL-2 is also involved in the regulation of the magnitude of the immune response and may contribute at the effector stage to long-term memory survival (Obar et al. 2010; Pipkin et al. 2010;

Williams et al. 2006). All three cytokines have been shown to regulate the expression of Bcl-2 in effector cells (Berard et al. 2003; Blattman et al. 2003; Tripathi et al. 2007), which was shown to be dependent on Stat5 (Tripathi et al. 2010). Utilizing CD127 mutant mice that cannot induce STAT5 phosphorylation in response to IL-7 stimulation, one study has shown that memory $CD8^+$ T cell homeostasis was impaired (Osborne et al. 2007). Although in vitro induction of Bcl-2 expression in response to IL-7 stimulation was normal, in vivo analysis showed low expression of Bcl-2 within T cells. Thus, there is clearly a requirement for IL-7-driven STAT5 activation to maintain normal levels of Bcl-2 and to promote memory $CD8^+$ T cell homeostasis in vivo.

Similar to naïve T cells and subsets of effector $CD8^+$ T cells, memory T cells are also maintained by a balance between Bim and Bcl-2 (Kurtulus et al. 2011; Wojciechowski et al. 2007). Whether other anti-apoptotic molecules play a role in the maintenance of memory T cells is not very clear as it is difficult to confine the effects to the memory stage. Conditional deletion of Bcl-xL in T cells did not suggest a role for Bcl-xL in the effector or memory T cells (Zhang and He 2005). The effects of Mcl-1 deletion in memory T cells could not be studied, as the survival of effector cells was severely impaired which did not allow tracking into the memory stage. Further work needs to be done using inducible knockout mice to determine the critical molecules required to promote memory T cell survival.

Bcl-2 Family Members in B Cell Development, Homeostasis, and Activation

B Cell Development

B cell development in the bone marrow is dependent on B cell receptor (BCR) and IL-7 receptor alpha (IL-7Rα) signaling. Like T cells, B cells undergo V(D)J recombination to form a functional BCR, a process that begins at the pro-B cell stage ($B220^+$ IgM^- $CD2^+$) with the rearrangement of the heavy Ig chain gene (Marsden and Strasser 2003). A properly formed pre-BCR provides critical survival signals, likely combating the pro-apoptotic proteins through Bcl-2 or Bcl-xL, as overexpression of Bcl-2 or Bcl-xL in $Rag^{-/-}$ and SCID mice rescues B cell development (Fang et al. 1996; Strasser et al. 1994a; Tarlinton et al. 1997). However, these mice also displayed reduced B cell maturation, demonstrating that pre-BCR and BCR signaling provides differentiation signals as well as survival signals (Tarlinton et al. 1997).

IL-7 is critical for B cell differentiation at the early stages of development, and in its absence B cell numbers are dramatically decreased in the bone marrow and peripheral lymphoid organs in mice. In the absence of IL-7 or IL-7Rα signaling, Bcl-2 overexpression or genetic deletion of Bim cannot rescue B cell development, specifically during the differentiation of common lymphoid progenitors (CLPs) to pro-B cells ($B220^+$ IgM^- $CD2^-$) and the conversion of pro-B cells into pre-B ($B220^+$ IgM^- $CD2^+$) and immature B cells ($B220^+$ IgM^+) (Maraskovsky et al. 1998; Oliver et al. 2004). However, the absence of Bim can rescue B cell development in the presence of limiting IL-7 (Huntington et al. 2009; Oliver et al. 2004). Thus, IL-7R signaling is required for more than just B cell survival during development; it is required for B cell differentiation by promoting expression of necessary transcription factors for B lineage commitment, such as early B cell factor (EBF) and Pax5, and by promoting BCR rearrangement (Corcoran et al. 1998; Dias et al. 2005; Miller et al. 2002).

The random rearrangement of the BCR is critical for the vast diversity of antigen receptors; however, the random antigen receptor generation allows for potential reactivity to self-antigen. Like T cells, B cells undergo negative selection during development to delete autoreactive cells, and this occurs during the transition from pre-B cell to immature B cell when the cells express a fully rearranged BCR. A double-transgenic mouse model expressing membrane-bound hen egg lysozyme (mHEL) and anti-HEL Ig is commonly used to study self-reactive B cells, and these mice have been used to elucidate the roles of intrinsic and extrinsic pathways of apoptosis. Using this model system, Rathmell and Goodnow

found that chronically HEL-stimulated B cells were eliminated by HEL-specific T cells, while naïve HEL-specific B cells were stimulated to undergo proliferation and antibody production after interaction with HEL-specific T cells (Rathmell et al. 1995). The former process was largely dependent upon Fas/FasL interactions. However, another group found that Bcl-2 overexpression rescued self-reactive B cells from death in vivo, indicating the mitochondrial pathway is involved in autoreactive B cell elimination (Hartley et al. 1993). Genetic deletion of Bim resulted in significantly more HEL reactive B cells in the periphery, albeit still less than mice that did not express the HEL antigen (Enders et al. 2003). These data suggest that either additional BH3-only proteins or Fas are partially redundant with Bim in eliminating autoreactive B cells or that there may be partial contributions of both Bim and Fas. Overall, both Bim and Fas may contribute to B cell central tolerance by mediating apoptotic driven elimination of autoreactive B cells.

B Cell Homeostasis

Immature B cells ($B220^+$ IgM^+) downregulate the expression of IL-7Rα and are no longer as dependent upon IL-7 for survival but instead are dependent on BAFF (B cell activating factor of the TNF family). BAFF is a cytokine critical for B cell activation, maturation, and survival in response to BCR signals and can break anergic B cell unresponsiveness (discussed below) (Batten et al. 2000; Ekland et al. 2004; Mackay and Browning 2002; Oliver et al. 2006). BAFF signals through the NF-κB pathway and has been shown to transiently increase Bcl-xL and A1 expression in mature B cells; however, A1 was not required for BAFF-induced survival (Hatada et al. 2003; Hsu et al. 2002). BAFF may also promote survival by downregulating Bim and increasing Bim phosphorylation through Erk activation (Craxton et al. 2005). Bim phosphorylation may regulate Bim activity by altering its binding to pro-apoptotic Bax (Harada et al. 2004) and/or anti-apoptotic Bcl-2 proteins. Thus, BAFF may function through several redundant mechanisms to counteract apoptosis in B cells.

B cells that escape negative selection during development may be regulated by anergy in the periphery. These anergic B cells have a reduced ability to respond to antigen, mature, and produce antibodies and exhibit a shortened lifespan. Similar to the mouse model of autoreactive B cell elimination, the mouse model for peripheral anergic B cells utilizes the anti-HEL Ig and soluble HEL (sHEL) double-transgenic mice. The Bcl-2 family members can influence the life of anergic B cells, as overexpression of Bcl-2 or Bcl-xL increased the survival of anergic B cells in the periphery, prolonged antibody responses, and elicited onset of autoimmune disease (Hartley et al. 1993; Strasser et al. 1991b). The genetic deletion of Bim in the anti-HEL/sHEL mouse model also increased peripheral B cell survival and promoted maturation of the anergic B cells (Oliver et al. 2006). This increased B cell maturation and increased autoantibody production was due to a reduced threshold requirement for BAFF-mediated maturation, thereby allowing the cells to break anergy (Oliver et al. 2006). Thus, Bcl-2 family members can affect peripheral tolerance/anergy by both affecting survival and BAFF-driven maturation thresholds in B cells.

B Cell Activation

Similar to T cells, B cells undergo expansion in response to an antigen, followed by a contraction of the B cell response and B cell memory development. One model used to look at BCR activation of B cells utilizes SpA superantigen, which binds a conserved V_H domain on BCR and induces activation independent of T cells and antigen processing (Goodyear et al. 2007). There is evidence that Bim mediates the death of superantigen-activated B cells, as Bim-deficient B cells are resistant to superantigen-induced death and exposure to BAFF in vitro ameliorated the superantigen-induced death of Bim-sufficient cells (Goodyear et al. 2007). Additional BAFF signaling may counteract Bim-mediated death by regulating Bim and Bcl-2 expression levels, as well as disrupting Bim-Bcl-2 complexes and inhibiting Bax/Bak activation, thereby promoting cell survival (Batten et al. 2000; Craxton et al. 2005). Bcl-2 is also critical for memory B cell

development in response to alum-adjuvant (NP-KLH) stimulation, as blocking of Bcl-2 by a pharmacological drug, ABT-737, inhibited the formation and maintenance of memory B cells (Carrington et al. 2010). However, whether these effects were due to direct effects of ABT-737 on B cells or were due to effects on T cell help were not clear. Overall, Bim and Bcl-2 may be the critical Bcl-2 family members involved in regulating activated B cell fate downstream of BAFF signaling, but further work is necessary to understand the particular molecules involved.

Conclusion

In summary, Bcl-2 family members are critically involved in different stages of lymphocyte survival. The interplay between Bim and Bcl-2 is the crucial factor in most of these stages. Regulation of downstream Bax/Bak molecules by Mcl-1 is also essential, but functions largely downstream of Bim and Bcl-2. Other Bcl-2 family members also are expressed in the lymphocyte lineage; their roles are either not decisive or function redundantly in lymphocyte survival.

Aberrant expression of anti-apoptotic Bcl-2 family members or loss of pro-apoptotic Bcl-2 molecules could result in loss of immune tolerance. Mouse models have shown that genetic deletion of Bim or overexpression of Bcl-2 can cause lymphoid neoplasia or late-onset spontaneous autoimmune diseases. Therefore, targeting Bcl-2 family members could prove to be useful in treatment of autoimmune diseases. There are BH3 mimetics available that can bind and inhibit a variety of anti-apoptotic Bcl-2 family members that may have therapeutic potential to prevent autoimmunity.

Cross-References

- ▶ Cytotoxic T Lymphocytes
- ▶ Fas/Fas Ligand
- ▶ T Cell Memory
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Akashi K, Kondo M, von Freeden-Jeffry U, Murray R, Weissman IL. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. Cell. 1997;89:1033–41.

Batten M, Groom J, Cachero TG, Qian F, Schneider P, Tschopp J, Browning JL, Mackay F. BAFF mediates survival of peripheral immature B lymphocytes. J Exp Med. 2000;192:1453–66.

Berard M, Brandt K, Bulfone-Paus S, Tough DF. IL-15 promotes the survival of naive and memory phenotype CD8+ T cells. J Immunol. 2003;170:5018–26.

Blattman JN, Grayson JM, Wherry EJ, Kaech SM, Smith KA, Ahmed R. Therapeutic use of IL-2 to enhance antiviral T-cell responses in vivo. Nat Med. 2003;9:540–7.

Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, Adams JM, Strasser A. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. Science. 1999;286:1735–8.

Bouillet P, Cory S, Zhang LC, Strasser A, Adams JM. Degenerative disorders caused by Bcl-2 deficiency prevented by loss of its BH3-only antagonist Bim. Dev Cell. 2001;1:645–53.

Bouillet P, Purton JF, Godfrey DI, Zhang LC, Coultas L, Puthalakath H, Pellegrini M, Cory S, Adams JM, Strasser A. BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. Nature. 2002;415:922–6.

Brunner T, Mogil RJ, LaFace D, Yoo NJ, Mahboubi A, Echeverri F, Martin SJ, Force WR, Lynch DH, Ware CF, et al. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature. 1995;373:441–4.

Carrington EM, Vikstrom IB, Light A, Sutherland RM, Londrigan SL, Mason KD, Huang DC, Lew AM, Tarlinton DM. BH3 mimetics antagonizing restricted prosurvival Bcl-2 proteins represent another class of selective immune modulatory drugs. Proc Natl Acad Sci USA. 2010;107:10967–71.

Chao DT, Korsmeyer SJ. BCL-XL-regulated apoptosis in T cell development. Int Immunol. 1997;9:1375–84.

Corcoran AE, Riddell A, Krooshoop D, Venkitaraman AR. Impaired immunoglobulin gene rearrangement in mice lacking the IL-7 receptor. Nature. 1998;391:904–7.

Craxton A, Draves KE, Gruppi A, Clark EA. BAFF regulates B cell survival by downregulating the BH3-only family member Bim via the ERK pathway. J Exp Med. 2005;202:1363–74.

Dhein J, Walczak H, Baumler C, Debatin KM, Krammer PH. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). Nature. 1995;373:438–41.

Dias S, Silva Jr H, Cumano A, Vieira P. Interleukin-7 is necessary to maintain the B cell potential in common lymphoid progenitors. J Exp Med. 2005;201:971–9.

Dunkle A, Dzhagalov I, He YW. Mcl-1 promotes survival of thymocytes by inhibition of Bak in a pathway separate from Bcl-2. Cell Death Differ. 2010;17:994–1002.

Dzhagalov I, Dunkle A, He YW. The anti-apoptotic Bcl-2 family member Mcl-1 promotes T lymphocyte survival at multiple stages. J Immunol. 2008;181:521–8.

Ekland EH, Forster R, Lipp M, Cyster JG. Requirements for follicular exclusion and competitive elimination of autoantigen-binding B cells. J Immunol. 2004;172:4700–8.

Enders A, Bouillet P, Puthalakath H, Xu Y, Tarlinton DM, Strasser A. Loss of the pro-apoptotic BH3-only Bcl-2 family member Bim inhibits BCR stimulation-induced apoptosis and deletion of autoreactive B cells. J Exp Med. 2003;198:1119–26.

Erlacher M, Labi V, Manzl C, Bock G, Tzankov A, Hacker G, Michalak E, Strasser A, Villunger A. Puma cooperates with Bim, the rate-limiting BH3-only protein in cell death during lymphocyte development, in apoptosis induction. J Exp Med. 2006;203:2939–51.

Fang W, Mueller DL, Pennell CA, Rivard JJ, Li YS, Hardy RR, Schlissel MS, Behrens TW. Frequent aberrant immunoglobulin gene rearrangements in pro-B cells revealed by a bcl-xL transgene. Immunity. 1996;4:291–9.

Fischer SF, Belz GT, Strasser A. BH3-only protein Puma contributes to death of antigen-specific T cells during shutdown of an immune response to acute viral infection. Proc Natl Acad Sci USA. 2008;105:3035–40.

Goodyear CS, Corr M, Sugiyama F, Boyle DL, Silverman GJ. Cutting edge: Bim is required for superantigen-mediated B cell death. J Immunol. 2007;178:2636–40.

Grillot DA, Merino R, Nunez G. Bcl-XL displays restricted distribution during T cell development and inhibits multiple forms of apoptosis but not clonal deletion in transgenic mice. J Exp Med. 1995;182:1973–83.

Hao Z, Duncan GS, Seagal J, Su YW, Hong C, Haight J, Chen NJ, Elia A, Wakeham A, Li WY, Liepa J, Wood GA, Casola S, Rajewsky K, Mak TW. Fas receptor expression in germinal-center B cells is essential for T and B lymphocyte homeostasis. Immunity. 2008;29:615–27.

Harada H, Quearry B, Ruiz-Vela A, Korsmeyer SJ. Survival factor-induced extracellular signal-regulated kinase phosphorylates BIM, inhibiting its association with BAX and proapoptotic activity. Proc Natl Acad Sci USA. 2004;101:15313–7.

Hartley SB, Cooke MP, Fulcher DA, Harris AW, Cory S, Basten A, Goodnow CC. Elimination of self-reactive B lymphocytes proceeds in two stages: arrested development and cell death. Cell. 1993;72:325–35.

Hatada EN, Do RK, Orlofsky A, Liou HC, Prystowsky M, MacLennan IC, Caamano J, Chen-Kiang S. NF-kappa B1 p50 is required for BLyS attenuation of apoptosis but dispensable for processing of NF-kappa B2 p100 to p52 in quiescent mature B cells. J Immunol. 2003;171:761–8.

Hildeman DA, Zhu Y, Mitchell TC, Bouillet P, Strasser A, Kappler J, Marrack P. Activated T cell death in vivo mediated by proapoptotic bcl-2 family member bim. Immunity. 2002;16:759–67.

Hildeman D, Jorgensen T, Kappler J, Marrack P. Apoptosis and the homeostatic control of immune responses. Curr Opin Immunol. 2007;19:516–21.

Hsu BL, Harless SM, Lindsley RC, Hilbert DM, Cancro MP. Cutting edge: BLyS enables survival of transitional and mature B cells through distinct mediators. J Immunol. 2002;168:5993–6.

Hughes PD, Belz GT, Fortner KA, Budd RC, Strasser A, Bouillet P. Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. Immunity. 2008;28:197–205.

Huntington ND, Labi V, Cumano A, Vieira P, Strasser A, Villunger A, Di Santo JP, Alves NL. Loss of the pro-apoptotic BH3-only Bcl-2 family member Bim sustains B lymphopoiesis in the absence of IL-7. Int Immunol. 2009;21:715–25.

Hutcheson J, Scatizzi JC, Bickel E, Brown NJ, Bouillet P, Strasser A, Perlman H. Combined loss of proapoptotic genes Bak or Bax with Bim synergizes to cause defects in hematopoiesis and in thymocyte apoptosis. J Exp Med. 2005;201:1949–60.

Hutcheson J, Scatizzi JC, Siddiqui AM, Haines 3rd GK, Wu T, Li QZ, Davis LS, Mohan C, Perlman H. Combined deficiency of proapoptotic regulators Bim and Fas results in the early onset of systemic autoimmunity. Immunity. 2008;28:206–17.

Jorgensen TN, McKee A, Wang M, Kushnir E, White J, Refaeli Y, Kappler JW, Marrack P. Bim and Bcl-2 mutually affect the expression of the other in T cells. J Immunol. 2007;179:3417–24.

Kawabe Y, Ochi A. Programmed cell death and extrathymic reduction of Vbeta8+ CD4+ T cells in mice tolerant to Staphylococcus aureus enterotoxin B. Nature. 1991;349:245–8.

Khaled AR, Kim K, Hofmeister R, Muegge K, Durum SK. Withdrawal of IL-7 induces Bax translocation from cytosol to mitochondria through a rise in intracellular pH. Proc Natl Acad Sci USA. 1999;96:14476–81.

Khaled AR, Li WQ, Huang J, Fry TJ, Khaled AS, Mackall CL, Muegge K, Young HA, Durum SK. Bax deficiency partially corrects interleukin-7 receptor alpha deficiency. Immunity. 2002;17:561–73.

Kim K, Lee CK, Sayers TJ, Muegge K, Durum SK. The trophic action of IL-7 on pro-T cells: inhibition of apoptosis of pro-T1, -T2, and -T3 cells correlates with Bcl-2 and Bax levels and is independent of Fas and p53 pathways. J Immunol. 1998;160:5735–41.

Kondrack RM, Harbertson J, Tan JT, McBreen ME, Surh CD, Bradley LM. Interleukin 7 regulates the survival and generation of memory CD4 cells. J Exp Med. 2003;198:1797–806.

Kurtulus S, Tripathi P, Opferman JT, Hildeman DA. Contracting the 'mus cells'–does down-sizing suit us for diving into the memory pool? Immunol Rev. 2010;236:54–67.

Kurtulus S, Tripathi P, Moreno-Fernandez ME, Sholl A, Katz JD, Grimes HL, Hildeman DA. Bcl-2 allows effector and memory CD8+ T cells to tolerate higher expression of Bim. J Immunol. 2011;186:5729–37.

Lenschow DJ, Sperling AI, Cooke MP, Freeman G, Rhee L, Decker DC, Gray G, Nadler LM, Goodnow CC, Bluestone JA. Differential up-regulation of the B7-1 and B7-2 costimulatory molecules after Ig receptor engagement by antigen. J Immunol. 1994;153:1990–7.

Li WQ, Jiang Q, Khaled AR, Keller JR, Durum SK. Interleukin-7 inactivates the pro-apoptotic protein Bad promoting T cell survival. J Biol Chem. 2004;279:29160–6.

Lindsten T, Ross AJ, King A, Zong WX, Rathmell JC, Shiels HA, Ulrich E, Waymire KG, Mahar P, Frauwirth K, Chen Y, Wei M, Eng VM, Adelman DM, Simon MC, Ma A, Golden JA, Evan G, Korsmeyer SJ, MacGregor GR, Thompson CB. The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. Mol Cell. 2000;6:1389–99.

Liston A, Lesage S, Gray DH, O'Reilly LA, Strasser A, Fahrer AM, Boyd RL, Wilson J, Baxter AG, Gallo EM, Crabtree GR, Peng K, Wilson SR, Goodnow CC. Generalized resistance to thymic deletion in the NOD mouse; a polygenic trait characterized by defective induction of Bim. Immunity. 2004;21:817–30.

Liu X, Zhu Y, Dai S, White J, Peyerl F, Kappler JW, Marrack P. Bcl-xl does not have to bind Bax to protect T cells from death. J Exp Med. 2006;203:2953–61.

Ma A, Pena JC, Chang B, Margosian E, Davidson L, Alt FW, Thompson CB. Bclx regulates the survival of double-positive thymocytes. Proc Natl Acad Sci USA. 1995;92:4763–7.

Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. Nat Rev Immunol. 2002;2:465–75.

Mandal M, Borowski C, Palomero T, Ferrando AA, Oberdoerffer P, Meng F, Ruiz-Vela A, Ciofani M, Zuniga-Pflucker JC, Screpanti I, Look AT, Korsmeyer SJ, Rajewsky K, von Boehmer H, Aifantis I. The BCL2A1 gene as a pre-T cell receptor-induced regulator of thymocyte survival. J Exp Med. 2005;201:603–14.

Maraskovsky E, O'Reilly LA, Teepe M, Corcoran LM, Peschon JJ, Strasser A. Bcl-2 can rescue T lymphocyte development in interleukin-7 receptor-deficient mice but not in mutant rag-1−/− mice. Cell. 1997;89:1011–9.

Maraskovsky E, Peschon JJ, McKenna H, Teepe M, Strasser A. Overexpression of Bcl-2 does not rescue impaired B lymphopoiesis in IL-7 receptor-deficient mice but can enhance survival of mature B cells. Int Immunol. 1998;10:1367–75.

Marsden VS, Strasser A. Control of apoptosis in the immune system: Bcl-2, BH3-only proteins and more. Annu Rev Immunol. 2003;21:71–105.

Merino D, Giam M, Hughes PD, Siggs OM, Heger K, O'Reilly LA, Adams JM, Strasser A, Lee EF, Fairlie WD, Bouillet P. The role of BH3-only protein Bim extends beyond inhibiting Bcl-2-like prosurvival proteins. J Cell Biol. 2009;186:355–62.

Miller JP, Izon D, DeMuth W, Gerstein R, Bhandoola A, Allman D. The earliest step in B lineage differentiation from common lymphoid progenitors is critically dependent upon interleukin 7. J Exp Med. 2002;196:705–11.

Motoyama N, Wang F, Roth KA, Sawa H, Nakayama K, Negishi I, Senju S, Zhang Q, Fujii S, et al. Massive cell death of immature hematopoietic cells and neurons in Bcl-x-deficient mice. Science. 1995;267:1506–10.

Obar JJ, Molloy MJ, Jellison ER, Stoklasek TA, Zhang W, Usherwood EJ, Lefrancois L. CD4+ T cell regulation of CD25 expression controls development of short-lived effector CD8+ T cells in primary and secondary responses. Proc Natl Acad Sci USA. 2010;107:193–8.

Oliver PM, Wang M, Zhu Y, White J, Kappler J, Marrack P. Loss of Bim allows precursor B cell survival but not precursor B cell differentiation in the absence of interleukin 7. J Exp Med. 2004;200:1179–87.

Oliver PM, Vass T, Kappler J, Marrack P. Loss of the proapoptotic protein, Bim, breaks B cell anergy. J Exp Med. 2006;203:731–41.

Opferman JT. Apoptosis in the development of the immune system. Cell Death Differ. 2008;15:234–42.

Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. Nature. 2003;426:671–6.

Osborne LC, Dhanji S, Snow JW, Priatel JJ, Ma MC, Miners MJ, Teh HS, Goldsmith MA, Abraham N. Impaired CD8 T cell memory and CD4 T cell primary responses in IL-7R alpha mutant mice. J Exp Med. 2007;204:619–31.

Pellegrini M, Bouillet P, Robati M, Belz GT, Davey GM, Strasser A. Loss of Bim increases T cell production and function in interleukin 7 receptor-deficient mice. J Exp Med. 2004;200:1189–95.

Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, Park LS, Ziegler SF, Williams DE, Ware CB, Meyer JD, Davison BL. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med. 1994;180:1955–60.

Petschner F, Zimmerman C, Strasser A, Grillot D, Nunez G, Pircher H. Constitutive expression of Bcl-xL or Bcl-2 prevents peptide antigen-induced T cell deletion but does not influence T cell homeostasis after a viral infection. Eur J Immunol. 1998;28:560–9.

Pipkin ME, Sacks JA, Cruz-Guilloty F, Lichtenheld MG, Bevan MJ, Rao A. Interleukin-2 and inflammation induce distinct transcriptional programs that promote

the differentiation of effector cytolytic T cells. Immunity. 2010;32:79–90.
Rathmell JC, Cooke MP, Ho WY, Grein J, Townsend SE, Davis MM, Goodnow CC. CD95 (Fas)-dependent elimination of self-reactive B cells upon interaction with CD4+ T cells. Nature. 1995;376:181–4.
Rathmell JC, Lindsten T, Zong WX, Cinalli RM, Thompson CB. Deficiency in Bak and Bax perturbs thymic selection and lymphoid homeostasis. Nat Immunol. 2002;3:932–9.
Rubinstein MP, Lind NA, Purton JF, Filippou P, Best JA, McGhee PA, Surh CD, Goldrath AW. IL-7 and IL-15 differentially regulate CD8+ T-cell subsets during contraction of the immune response. Blood. 2008;112:3704–12.
Steimer DA, Boyd K, Takeuchi O, Fisher JK, Zambetti GP, Opferman JT. Selective roles for antiapoptotic MCL-1 during granulocyte development and macrophage effector function. Blood. 2009;113:2805–15.
Strasser A. The role of BH3-only proteins in the immune system. Nat Rev Immunol. 2005;5:189–200.
Strasser A, Harris AW, Cory S. bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell. 1991a;67:889–99.
Strasser A, Whittingham S, Vaux DL, Bath ML, Adams JM, Cory S, Harris AW. Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. Proc Natl Acad Sci USA. 1991b;88:8661–5.
Strasser A, Harris AW, Corcoran LM, Cory S. Bcl-2 expression promotes B- but not T-lymphoid development in scid mice. Nature. 1994a;368:457–60.
Strasser A, Harris AW, von Boehmer H, Cory S. Positive and negative selection of T cells in T-cell receptor transgenic mice expressing a bcl-2 transgene. Proc Natl Acad Sci USA. 1994b;91:1376–80.
Surh CD, Sprent J. Homeostasis of naive and memory T cells. Immunity. 2008;29:848–62.
Tarlinton DM, Corcoran LM, Strasser A. Continued differentiation during B lymphopoiesis requires signals in addition to cell survival. Int Immunol. 1997;9:1481–94.
Tomayko MM, Punt JA, Bolcavage JM, Levy SL, Allman DM, Cancro MP. Expression of the Bcl-2 family member A1 is developmentally regulated in T cells. Int Immunol. 1999;11:1753–61.
Tripathi P, Mitchell TC, Finkelman F, Hildeman DA. Cutting edge: limiting amounts of IL-7 do not control contraction of CD4+ T cell responses. J Immunol. 2007;178:4027–31.
Tripathi P, Kurtulus S, Wojciechowski S, Sholl A, Hoebe K, Morris SC, Finkelman FD, Grimes HL, Hildeman DA. STAT5 is critical to maintain effector CD8+ T cell responses. J Immunol. 2010;185:2116–24.
Williams MA, Tyznik AJ, Bevan MJ. Interleukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells. Nature. 2006;441:890–3.
Wojciechowski S, Jordan MB, Zhu Y, White J, Zajac AJ, Hildeman DA. Bim mediates apoptosis of CD127(lo) effector T cells and limits T cell memory. Eur J Immunol. 2006;36:1694–706.
Wojciechowski S, Tripathi P, Bourdeau T, Acero L, Grimes HL, Katz JD, Finkelman FD, Hildeman DA. Bim/Bcl-2 balance is critical for maintaining naive and memory T cell homeostasis. J Exp Med. 2007;204:1665–75.
Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol. 2008;9:47–59.
Zhang N, He YW. The antiapoptotic protein Bcl-xL is dispensable for the development of effector and memory T lymphocytes. J Immunol. 2005;174:6967–73.
Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. Genes Dev. 2001;15:1481–6.

BCR Signaling

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Synonyms

B cell receptor (BCR) signaling; BCR signal transduction; BCR signaling cascade

Definition

BCR signaling refers to the signal(s) that the B cell receptor transmits into a B cell from the cell surface. This signal is translated into various activities ranging from gene transcription, protein expression, cell survival, mitosis, apoptosis, etc., depending on the type of signal and the context. Two types of BCR signaling have been identified: a basal (also known as tonic) that appears to be ligand-independent, and an antigen-induced that is generated upon the receptor binding to cognate antigen.

Introduction

The B cell receptor and its signaling function are necessary for the development, function, and survival of B cells (Conley et al. 2009). These cells are specialized in making antibodies that react with any type of molecule. With their enhanced specificity and reactivity and their capacity for modulating the activity of other blood cell types, B cells are an effector component of the adaptive immune system and, as such, their development, selection, and activation require proper regulation. This regulation is partly imparted by BCR signaling. BCR signaling has multiple roles depending on the maturation stage of the B cell. During B cell development, BCR signaling guides the maturation of B cells from the B cell precursor stage in the bone marrow to the mature B cell stage in peripheral tissue (Thomas et al. 2006). Genetic mutations that preclude appropriate BCR signaling lead to B cell deficiencies and hypogammaglobulinemia. Between the immature and mature developmental B cell stages, BCR signaling is also important for tolerance induction, directing the selection of B cells such that the majority of cells within the mature B cell pool are specific for foreign molecules and only a minority reacts with self-antigens (Pelanda and Torres 2012). Thus, defects in BCR signaling can result in higher numbers of autoreactive B cells with the potential for autoimmunity. Within mature naive B cells, basal BCR signaling functions to maintain their survival, while antigen-induced BCR signaling promotes their activation and differentiation into effector cells such as memory B cells and antibody-forming cells (plasmablasts and plasma cells) (Thomas et al. 2006; Mackay et al. 2010). Overall, therefore, BCR signaling controls the formation of the B cell and antibody repertoire.

Structural Components of the BCR

The BCR is composed of four B cell–specific proteins: immunoglobulin (Ig) heavy and Ig light chains and the Ig-α (CD79A) and Ig-β (CD79B) polypeptides (Reth et al. 2000; Kurosaki 2011). The Ig heavy and light chains (two each per receptor) pair to form an immunoglobulin or antibody that serves as the antigen-specific portion of the BCR. Each B cell expresses unique Ig heavy and light chain variable sequences that convey the cell specificity for an antigen; the Ig heavy chain contains in addition a transmembrane region that anchors the Ig in the plasma membrane. One Ig-α/Ig-β heterodimer associates with one of the Ig heavy chain in each BCR to form its signaling portion. All four proteins must be expressed for proper BCR assembly and translocation onto the cell surface from where the receptor signals into the cell. Ig-α and Ig-β are transmembrane proteins that contain Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) within their respective cytoplasmic portions. The tyrosine residues within the ITAMs are absolutely required for the transmission of the BCR signaling inside the cell and are the main signaling components of the BCR (Kraus et al. 2001). The Ig heavy chain can also participate in BCR signaling depending on the constant region they display and, specifically, the length of their cytoplasmic tails. While μ and δ constant regions (IgM and IgD receptors) have short (3 amino acids) cytoplasmic tails that are unable to signal, γ, ε, and α (IgG, IgE, and IgA receptors) exhibit extended tails with conserved tyrosines that contribute to BCR signaling.

Signal Initiation

BCR signaling begins when the tyrosine residues of Ig-α/Ig-β ITAMs are phosphorylated by tyrosine kinases (Yang and Reth 2010; Kurosaki 2011). This protein modification activates the BCR signaling cascade inside the cell, but the extent of this signal transduction event depends on the level of ITAM phosphorylation in each receptor and between receptors. Studies performed prevalently in cell lines indicate that the BCR is constitutively associated with tyrosine kinases that, in a naive state (i.e., in the absence of BCR stimulation), display low phosphorylation activities. These basal activities have been proposed to result in a low level of BCR phosphorylation that initiates and maintains the activity of

the tonic or basal BCR signaling cascade (Pierce and Liu 2010). Upon binding antigen, the BCR is rapidly and extensively phosphorylated, an event associated with full activation of the receptor proximal tyrosine kinases and receptor clustering, and leading to the activation of the BCR signaling cascade and its biological consequences. The precise manner by which BCR signal transduction is initiated is not yet fully understood. Relocation of the BCR into specialized plasma membrane regions, specific interactions with the cytoskeleton, and conformational changes are some of the mechanisms under consideration (Harwood and Batista 2010; Pierce and Liu 2010; Yang and Reth 2010).

The tyrosine kinases responsible for the phosphorylation of the BCR and the initiation of BCR signal transduction are the Src family kinases (mainly Lyn, Blk, and Fyn) and the Syk family kinases (mainly Syk). Genetic mutations that lead to the deletion or inactivation of these kinases have severe consequences on B cell development and function, indicating their prominent role in B cell biology (Xu et al. 2005; Conley et al. 2009; Mocsai et al. 2010).

The BCR Signaling Cascade

The BCR proximal tyrosine kinases of the Src and Syk families contain Src homology 2 (SH2) domains with which they dock to the BCR via binding to the phosphorylated tyrosines (Cambier and Getahun 2010; Yang and Reth 2010; Kurosaki 2011). Syk, in particular, contains tandem SH2 domains that dock to the two phosphotyrosines in the ITAM. This binding leads to further activation of the Src and Syk kinases and the recruitment and/or activation of additional elements of the BCR signaling cascade including the BLNK/SLP-65 and BCAP adaptor proteins, the Tec family kinase Btk, and the Vav guanine nucleotide exchange factors, which are all phosphorylated by Syk (Mocsai et al. 2010). Activation of these proximal elements of the BCR signaling cascade leads to activation of further downstream signaling pathways including PLC-γ, Ras, PI3K, and Rac/cdc42 (Thomas et al. 2006; Mackay et al. 2010; Baracho et al. 2011; Kurosaki 2011). The phospholipase C-γ (PLC-γ), an enzyme that hydrolyzes phosphatidylinositol-4,5-biphosphate (PIP_2) into inositol triphosphate (IP_3) and diacylglycerol (DAG), induces extracellular Ca^{++} influx and activation of the NFAT and NF-κB-mediated gene transcriptional programs. The small GTPase Ras family members not only induce the activation of the Mitogen-Activated Protein Kinase (MAPK) Erk pathway, but also contribute to the activation of PI3K and other pathways. The phosphatidylinositide-3-kinase (PI3K) promotes the activation of the Akt and mTOR downstream pathways, while the Rho family GTPase Rac can lead to the activation of the MAPK JNK pathway. Significant cross-modulation exists between these pathways and it involves several types of protein modifications, the most prevalent of which are tyrosine, serine, and threonine phosphorylation (activating or inhibiting depending on the specific protein domain); ubiquitination leading to protein degradation; tyrosine, serine and threonine dephosphorylation by phosphatases; and GTP/GDP exchanges. Downstream pathways of BCR signaling involve DNA binding factors (such as NFAT, NF-κB, Erk, and JNK) that translocate into the nucleus to switch on, switch off, or modulate the transcription of several genes that control changes at the cellular level.

Consequences of BCR Signaling

Tonic BCR signaling in immature B cells leads to the basal activation of the Erk and the PI3K pathways and to the expression of the BAFF cytokine receptor BAFFR that initiates its own signal transduction upon binding BAFF (Werner et al. 2010; Pelanda and Torres 2012). These pathways promote the further differentiation of immature B cells into mature B cells and inhibit Ig gene recombination, establishing allelic exclusion and monoclonal antibody expression. In mature naive B cells, tonic BCR signaling is essential for cell survival, a process mediated via the activities of the

PI3K-Akt pathway and the increased expression of prosurvival Bcl-2 family members (Mackay et al. 2010; Werner et al. 2010; Baracho et al. 2011).

Antigen-mediated BCR signaling has more diverse cellular consequences, depending on the stage of B cell maturation and the type of costimulation. A consequence of antigen-induced BCR signaling is receptor internalization, which in mature B cells provides a mean for internalizing and processing antigens and then present antigen-derived peptides to T cells (Harwood and Batista 2010). At the immature B cell stage of B cell development, the stage at which B cells first express surface IgM, antigen-induced BCR signaling leads to B cell tolerance. This is a process evolved to revise (i.e., receptor editing), eliminate (i.e., clonal deletion), or inactivate (i.e., anergy) B cells that react with self-antigens, thus promoting the formation of a mature B cell pool that is only minimally autoreactive (Shlomchik 2008; Pelanda and Torres 2012). Low levels of antigen-induced BCR signaling, however, are compatible with selection of immature B cells into the mature B cell pool and, in fact, direct entry into specific mature B cell subsets such as the follicular (no signal) and the B1 and marginal zone (low signal) B cell subsets (Thomas et al. 2006).

At the mature B cell stage, antigen-induced BCR signaling causes either B cell tolerance or B cell activation and these differential outcomes depend on the presence of costimulation (Shlomchik 2008; Goodnow et al. 2010). Upon BCR signaling but in the absence of additional stimuli, B cells may undergo initial blasting, but do not further progress in activation and differentiation. In fact, if the BCR signaling is chronic, such as that mediated by a self-antigen, the B cell becomes anergic (i.e., nonfunctional) and eventually dies. In the presence of signals mediated by certain co-receptors, on the other hand, B cells undergo extensive activation characterized by high levels of CD86, CD69, and MHC class II expression. Moreover, these cells undergo proliferation and clonal expansion, and also differentiation into antibody-forming cells and memory B cells. Stimuli that lead to full activation of antigen-binding B cells are those coming from T cells that react with peptides presented by the B cells within the context of a cognate (i.e., antigen specific) interaction. During these close encounters, T cell–expressed CD28 and CD154 bind the CD80/CD86 (also known as B7-1/B7-2) and CD40 co-receptors on B cells, respectively, and IL-4 produced by T cells binds to the B cell IL-4 receptor, and these ligands induce signals essential for B cell activation. Additional T cell-derived signals (e.g., IL-21) further contribute to the successful participation of B cells in germinal center reactions. These reactions include somatic hypermutation, affinity maturation, and class switch recombination of the Ig genes, which lead to the expression of IgG, IgA, and IgE antibodies with higher affinity for the selective antigen. Furthermore, germinal center reactions lead to the differentiation of B cells into memory B cells and short- and long-lived plasma cells.

Antigen-stimulated B cells can also undergo activation and differentiation in the presence of T cell–independent signals (Rawlings et al. 2012; Swanson et al. 2013). For instance, microbe-specific molecules can bind to Toll-like receptors (TLRs) on the surface of B cells, and TLR signaling synergizes with BCR signaling to induce B cell proliferation and B cell differentiation into short-lived plasmablasts and plasma cells.

Modulation and Termination of BCR Signaling

BCR signaling can be further modulated by co-receptor molecules in either a positive or negative manner. Some of these co-receptors are CD19, CD21/35, CD22, CD32 (FcγRIIB), and CD72. Among these, CD19 and FcγRIIB deserve a special mention. CD19 is a positive regulator of BCR signaling and acts at many stages of B cell maturation, during B cell development in the bone marrow and in antigen-specific B cell responses in peripheral tissue (Del Nagro et al. 2005). Tyrosine residues in the CD19 chain are phosphorylated upon BCR signaling mainly by the Src family kinases Lyn and Fyn. These phosphorylation events recruit and activate PI3K, thus

mediating some of the cellular changes associated with the PI3K pathway in BCR-stimulated B cells (Baracho et al. 2011). Moreover, CD19 together with CD21 is involved in the binding of complement, positively modulating BCR signaling in B cells that bind antigen in complex with complement (Del Nagro et al. 2005).

FcγRIIB, an inhibitory member of the Fcγ receptor family, modulates B cell activation upon binding the Igγ constant region of IgG-antigen immune complexes (Nimmerjahn and Ravetch 2010). FcγRIIB signal transduction involves the activation of the tyrosine and phosphatidylinositol phosphatases SHP-1 and SHIP-1, respectively (Cady et al. 2008). These phosphatases decrease the activity of the BCR signaling cascade, negatively modulating B cell activation. In the presence of antigen-specific circulating IgG, a B cell that simultaneously binds immune complexes via the BCR and the FcγRIIB receives both positive (BCR) and negative (FcγRIIB) signals. Only B cells with high affinity for antigen retain enough positive BCR signaling to continue their activation. Thus, CD19 and FcγRIIB play an important role in antibody responses, such that both low affinity cross-reactive as well as high affinity and highly specific antibodies are represented within the repertoire.

Termination of BCR signaling is needed to prevent sustained activation of B cells once the antigen has been removed. Phosphatases such as the SHP-1 and SHIP-1 described above are largely responsible for the termination of BCR signaling that follows antigen-mediated activation, as indicated by the pathological activation of B cells in mice that lack these enzymes (Cambier and Getahun 2010; Waterman and Cambier 2010). However, many other feedback inhibitory pathways exist along the BCR signaling cascade and that ensure that each of the signaling mediators is recalibrated to the baseline activity.

Conclusion

BCR signaling is an indispensable biological process for the generation, survival, and activation of B cells and is, therefore, of crucial importance for adaptive and, particularly, humoral immune responses. BCR signaling is initiated by the BCR, is mediated by a variety of intracellular molecules, and is modulated by several co-receptors. Given its role in regulating B cell development and activation, genetic polymorphisms and mutations that affect its function can result in a broad array of symptoms and diseases including immunodeficiency and autoimmunity.

Cross-References

- B Cell Tolerance
- B7 and CD28 Families
- CD40
- Immunodeficiency in Autoimmune Diseases
- Mammalian Target of Rapamycin (mTOR)
- Nuclear Factor of Activated T Cells (NFAT)
- NF-κB
- PI3K
- Rho/Rac GTPases
- SH2 Domain-containing Inositol Phosphatase-1 (SHIP)

References

Baracho GV, Miletic AV, et al. Emergence of the PI3-kinase pathway as a central modulator of normal and aberrant B cell differentiation. Curr Opin Immunol. 2011;23(2):178–83.

Cady CT, Rice JS, et al. Regulation of hematopoietic cell function by inhibitory immunoglobulin G receptors and their inositol lipid phosphatase effectors. Immunol Rev. 2008;224:44–57.

Cambier JC, Getahun A. B cell activation versus anergy; the antigen receptor as a molecular switch. Immunol Lett. 2010;128(1):6–7.

Conley ME, Dobbs AK, et al. Primary B cell immunodeficiencies: comparisons and contrasts. Annu Rev Immunol. 2009;27:199–227.

Del Nagro CJ, Otero DC, et al. CD19 function in central and peripheral B-cell development. Immunol Res. 2005;31(2):119–31.

Goodnow CC, Vinuesa CG, et al. Control systems and decision making for antibody production. Nat Immunol. 2010;11(8):681–8.

Harwood NE, Batista FD. Early events in B cell activation. Annu Rev Immunol. 2010;28:185–210.

Kraus M, Pao LI, et al. Interference with immunoglobulin (Ig)alpha immunoreceptor tyrosine-based activation

motif (ITAM) phosphorylation modulates or blocks B cell development, depending on the availability of an Igbeta cytoplasmic tail. J Exp Med. 2001;194(4):455–69.
Kurosaki T. Regulation of BCR signaling. Mol Immunol. 2011;48(11):1287–91.
Mackay F, Figgett WA, et al. B-cell stage and context-dependent requirements for survival signals from BAFF and the B-cell receptor. Immunol Rev. 2010;237(1):205–25.
Mocsai A, Ruland J, et al. The SYK tyrosine kinase: a crucial player in diverse biological functions. Nat Rev Immunol. 2010;10(6):387–402.
Nimmerjahn F, Ravetch JV. Antibody-mediated modulation of immune responses. Immunol Rev. 2010;236:265–75.
Pelanda R, Torres RM. Central B-cell tolerance: where selection begins. Cold Spring Harb Perspect Biol. 2012;4(4):a007146.
Pierce SK, Liu W. The tipping points in the initiation of B cell signalling: how small changes make big differences. Nat Rev Immunol. 2010;10(11):767–77.
Rawlings DJ, Schwartz MA, et al. Integration of B cell responses through Toll-like receptors and antigen receptors. Nat Rev Immunol. 2012;12(4):282–94.
Reth M, Wienands J, et al. An unsolved problem of the clonal selection theory and the model of an oligomeric B-cell antigen receptor. Immunol Rev. 2000;176:10–8.
Shlomchik MJ. Sites and stages of autoreactive B cell activation and regulation. Immunity. 2008;28(1):18–28.
Swanson CL, Pelanda R, et al. Division of labor during primary humoral immunity. Immunol Res. 2013;55(1–3):277–86.
Thomas MD, Srivastava B, et al. Regulation of peripheral B cell maturation. Cell Immunol. 2006;239(2):92–102.
Waterman PM, Cambier JC. The conundrum of inhibitory signaling by ITAM-containing immunoreceptors: potential molecular mechanisms. FEBS Lett. 2010;584(24):4878–82.
Werner M, Hobeika E, et al. Role of PI3K in the generation and survival of B cells. Immunol Rev. 2010;237(1):55–71.
Xu Y, Harder KW, et al. Lyn tyrosine kinase: accentuatingthe positive and the negative. Immunity. 2005;22(1):9–18.
Yang J, Reth M. The dissociation activation model of B cell antigen receptor triggering. FEBS Lett. 2010;584(24):4872–7.

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Cancer and Dermatomyositis

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Synonyms

Amyopathic dermatomyositis; Idiopathic inflammatory myopathy; Juvenile Dermatomyositis; Polymyositis

Definition

Dermatomyositis – Inflammation of the skin and underlying muscle tissue, typically occurring as an autoimmune condition or associated with internal cancer.

Introduction

Dermatomyositis (DM) is an acquired skeletal muscle disease with characteristic skin and extramuscular manifestations and is generally classified as an idiopathic inflammatory myopathy. Though DM has a wide range of phenotypic presentations and clinical overlap with other idiopathic inflammatory myopathies, including polymyositis (PM) and inclusion body myositis (IBM), DM is a distinct disorder with a unique pathophysiology. This entry will review the clinical signs and symptoms, workup, treatment, and prognosis of DM and its subtypes. The immunopathology and pathophysiology of DM is emphasized within the entry, as there has been a rapidly growing body of literature in these areas.

Epidemiology

Dermatomyositis can affect a wide age group with two peaks of onset, including 5–10 years of age and 30–50 years of age. The incidence is roughly 1 per 100,000 annually (Dalakas and Hohlfeld 2003), and it is about twice as common in women than men. There is no racial predilection.

Clinical Signs/Symptoms

Given the considerable clinical overlap between the idiopathic inflammatory myopathies, neuromuscular disorders, and many connective tissue disorders, definitive diagnosis has long been a challenging issue. In 1975, Bohan and Peter created a set of diagnostic criteria for polymyositis and dermatomyositis that included clinical symptoms and signs, muscle pathology, serum biochemical markers, and electromyographic data. With the advent of more advanced

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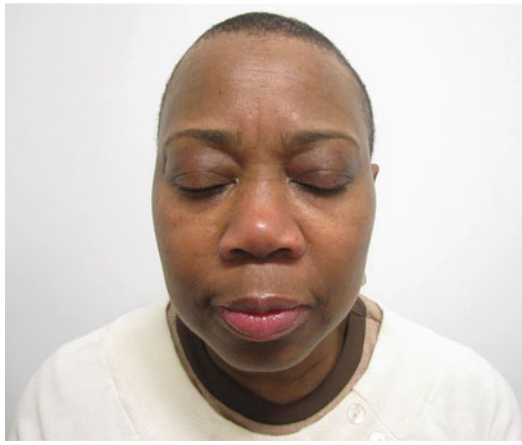
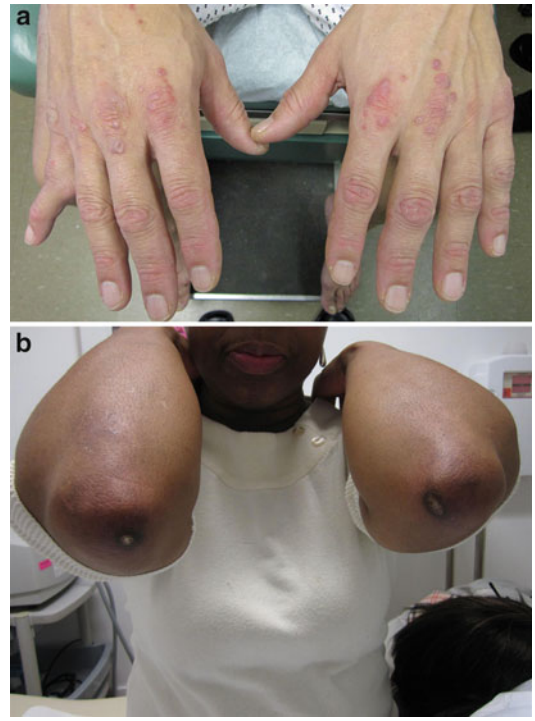


Cancer and Dermatomyositis, Fig. 1 A violaceous hue with periorbital edema is consistent with heliotrope sign of dermatomyositis (Source: Dr. George I. Varghese)



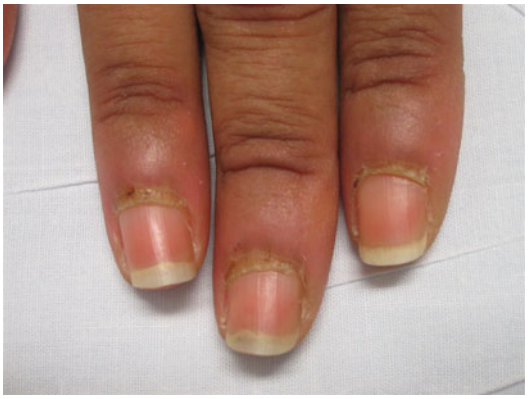
Cancer and Dermatomyositis, Fig. 2 (**a**) Gottron's papules are seen prominently on the MCP joints of the dorsal hands (**b**) Gottron's papules can also be found on the elbows as seen in this African American woman (Source: Dr. George I. Varghese)

immunological research, these criteria have since been modified (Callen 2000; Dalakas and Hohlfeld 2003).

Clinically, the most salient feature that distinguishes DM from other inflammatory myopathies is the skin involvement. It is often the first manifestation and, in a subset of people with DM, can be the sole manifestation as well (Callen 2000). The heliotrope rash, a violaceous macular erythema symmetrically involving the periorbital skin, with or without underlying edema, is pathognomonic for DM (Fig. 1). It may be subtle, only involving the eyelid margin. Gottron's papules, also specific for DM, are raised violaceous papules and plaques with a scaly eruption over the bony prominences of the body, especially the metacarpophalangeal joints (Fig. 2). Poikiloderma is another common feature found in DM that consists of hyperpigmented, variegated, telangiectasias along photosensitive areas of skin, often along the upper chest and back (the "shawl sign").

Other nonspecific cutaneous features that may be seen in DM include malar erythema, violaceous erythema along extensor surfaces, and psoriasiform erythema along the scalp, often associated with non-scarring alopecia (Callen 2000). Telangiectasias at the base of the fingernails and cuticular hypertrophy are common in DM (Fig. 3). Cutaneous lesions less characteristic of DM, but reported to occur, include panniculitis, urticaria, hyperkeratosis of the palms causing a rough and cracked appearance (mechanic's hands), ichthyosis, follicular hyperkeratosis, oral mucosal lesions, and, rarely, erosive vesiculobullous lesions and exfoliative erythroderma (Callen 2000).

Usually, skin findings occur prior to the development of muscle weakness, though it should be noted that this is not an absolute: muscle disease may occur concurrently or even before a rash is evident (Dalakas and Hohlfeld 2003). Muscle weakness is characteristically symmetric, proximal, and slowly progressive. It is often noted by patients as fatigue when performing certain actions, such as climbing stairs, rising from a sitting position, combing one's hair, or reaching for items above one's shoulders. Pain and tenderness in the muscles is actually not commonly seen, occurring in less than 30 % of patients (Dalakas and Hohlfeld 2003).



Cancer and Dermatomyositis, Fig. 3 Thickening of the proximal nail fold with telangiectasias can give an appearance of "ragged" cuticles in patients with dermatomyositis (Source: Dr. George I. Varghese)

A subset of DM, called amyopathic dermatomyositis or dermatomyositis sine myositis, includes cases in which there are typical skin findings of DM but an absence of muscle weakness on exam or history. Ten to twenty percent of patients with DM are amyopathic, and some experts believe that this may actually underestimate the true number (Gerami et al. 2006). The most common associated symptoms are lethargy, fatigue, arthralgias, and photosensitivity (Euwer and Sontheimer 1991). Some researchers contend that there is subclinical muscle involvement in these patients seen on EMG, biopsy, or imaging (e.g., MRI, ultrasound, spectroscopy), suggesting that this subset is in reality just part of the mild end of phenotypic variance seen in dermatomyositis (Dalakas and Hohlfeld 2003). It is difficult to say for certain, however, as patients with no clinical muscle involvement generally do not get further testing and are often diagnosed with amyopathic dermatomyositis on a clinical basis alone. As a practical point, Euwer and Sontheimer (1991) do point out that these tests are not indicated, as they do not affect treatment.

Juvenile dermatomyositis looks, for the most part, like the adult-onset form, though certain characteristics of the disease do set it apart, aside from age of onset (defined as below 18 years of age). Clinical symptoms in children often have an insidious onset and can be nonspecific, including fatigue, irritability, arthralgias, pruritus, and facial flushing. In one study, an extremity rash (usually violaceous to erythematous scaly plaques on extensor surfaces) was the most common initial presentation, followed by the heliotrope rash (Peloro et al. 2001). This study also showed that, in children, scalp dermatitis might be more common than previously thought, affecting 25 % of the cohort studied. Flexion contracture of the ankles, causing a tiptoe gait, is common (Dalakas and Hohlfeld 2003). Calcinosis is a frequent complication in up to 40 % of children but may not develop until later in the course, and might be prevented with early aggressive therapy (Callen 2000). The incidence of malignancy in juvenile DM is much lower than adult DM (Hiketa et al. 1992). An amyopathic form of DM may occur in children as well (Gerami et al. 2007).

There are myriad systemic manifestations of dermatomyositis, many of which are shared with polymyositis. General symptoms may include fever, malaise, and arthralgias. Joints are commonly affected, and symptoms can range from nonerosive joint swelling to joint contractures. Raynaud's phenomenon can be seen with DM. Dysphagia and dysphonia can occur due to involvement of the striated muscles of the oropharynx and upper esophagus. Cardiac findings include conduction defects, dysrhythmias, and myocarditis. Respiratory symptoms may occur from weakness of the thoracic muscles, interstitial lung disease, or pneumonitis and may occur in 15–30 % of patients (Callen 2000). Calcinosis of the subcutaneous tissue is more common in children with DM. On exam, calcifications are seen as firm, yellow, or flesh-colored nodules over bony protuberances; in some cases, they can extrude through the skin, causing pain and ulceration. Rarely, a fulminant clinical course can develop consisting of one or more of the following: diffuse alveolar damage, rapidly progressive interstitial pneumonitis, adult respiratory distress syndrome, and severe vasculopathy causing cutaneous, cerebral, and gastrointestinal infarcts (Magro et al. 2009).

Etiology

The etiology of DM remains unknown and likely multifactorial. Studies have demonstrated a genetic component through an association with certain HLA genes (Dalakas and Hohlfeld 2003), and a tumor necrosis factor polymorphism linked to photosensitivity (Werth et al. 2002). Autoantibodies are certainly linked to inflammatory myopathies, though their etiological significance is uncertain. Viral triggers have been postulated and may interact with an immunogenetic predisposition for developing DM, but no clear proof of causation has been established (Callen and Wortmann 2006; Magro et al. 2009). Some medications including hydroxyurea, quinidine, and penicillamine have been noted to trigger DM-like symptoms (Callen 2000).

Immunopathophysiology

The pathogenesis of dermatomyositis has been studied and discussed, but not fully elucidated, given the complex nature of a disease affecting multiple organ systems. There appears to be both immune and nonimmune-mediated destruction of muscle fibers as well as ischemic vasculopathic changes in both muscle and skin.

To understand DM pathophysiology, it is important to understand what is seen histopathologically. Myofiber alteration with microvascular and secondary ischemic changes provide the basis of the pathology seen in patients with dermatomyositis (Greenberg 2008). Muscle biopsies in DM show perifascicular atrophy and a pauci-inflammatory response surrounding myofibers. Within the perifascicular areas are microinfarcts, consisting of small foci of contiguous necrotic or regenerating fibers. Focal loss of myosin within the perifascicular areas gives the myofibers a "honeycomb-like" appearance. Further studies have shown significant vascular damage playing a role in DM. Capillary damage and dropout occurs early within the perifascicular areas and may be due to a number of factors including endothelial hyperplasia due to abnormal filamentous structures, known as tubuloreticular inclusions, found within capillary endothelial cells, as well as early deposition of complement C5b-9 membranolytic attack complex (MAC) on endothelial cells (Kissel et al. 1986). Inflammatory infiltrates composed of mononuclear cells, including T lymphocytes, B lymphocytes, and macrophages, also contribute to the immune-mediated response in the muscle.

Immune and Nonimmune Pathways in DM Pathogenesis

Adaptive Immune System

The adaptive immune system, composed of the humoral and cell-mediated immune response, has been strongly implicated in DM largely due to the numerous antibodies that bind to specific autoantigens of DM, the presence of an inflammatory infiltrate, and its associations with HLA-DR genotypes and other autoimmune and vascular diseases.

Up to 80 % of patients with either DM or PM have specific autoantibodies or antinuclear antibodies. There are a number of specific antibodies that directly relate to distinct clinical features of the disease. Anti-helicase (Mi2) antibodies show a strong association with the cutaneous findings of DM, including Gottron's papules, heliotrope rash, V-sign, and the shawl sign (Targoff 2006). Aminoacyl transfer RNA (tRNA) synthetases are specific to myositis and are associated with a distinct clinical entity known as antisynthetase syndrome. Its features include myositis, interstitial lung disease, nonerosive arthritis, Raynaud's phenomenon, and mechanic's hands. To date, eight different antisynthetase antibodies have been isolated, the most common being anti-histidyl-tRNA synthetase (anti-Jo1). All the antisynthetase antibodies share a common clinical feature, interstitial lung disease. There are other antibodies that are not specific, but associated with myositis, including anti-snRNP, anti-Ro/SSa, anti-Ku, and anti-PMS1 (Nagaraju and Lundberg 2011).

The humoral immune response is quite prevalent given the number of circulating B cells and plasma cell infiltrates found within muscle in patients with myositis. Antigens, whether

autoantigens or exogenous cross-antigens (viruses or other infectious agents), localized to the muscle may potentially drive a B cell antigen-specific response in myositis (Bradshaw et al. 2007). The pathogenesis of these specific autoantibodies is still unclear because these are not muscle-specific autoantibodies. Rather, they are directed toward ubiquitous intracellular proteins. However, there is evidence that the autoantibodies do show differential organ expression, specifically with histidyl-tRNA synthetase, where there is a higher expression in the epithelial cells of the bronchi related to other healthy organs and conformations within the lung suggesting autoimmunity to histidyl-tRNA synthetase (Levine et al. 2007).

The cell-mediated response occurs through two lymphocytic pathways that drive the immune response in muscle tissue. The first pathway is composed of CD4+ T lymphocytes, B lymphocytes, macrophages, plasma cells, and dendritic cells predominately found in perivascular and perimysial areas of tissue (Dalakas and Hohlfeld 2003). This pathway is primarily directed toward blood vessels creating a vasculopathy. The capillaries are predominately affected as a result of immune-mediated complement deposition in the vessels. Capillaries within muscles decrease in size and may show hyperplasia and necrosis, leading to ischemic changes and subsequent muscle damage. Clinically, the pathology of this pathway is manifested through nail fold telangiectasias seen in DM. The second pathway is dominated by CD8+ T lymphocytes, CD4+ T lymphocytes, macrophages, and dendritic cells found in an endomysial distribution, with inflammatory cells invading non-necrotic muscle fibers (Dalakas and Hohlfeld 2003). This pathway is directed exclusively to muscle fibers and is usually found in patients with myositis without skin rash or polymyositis. CD8+ T lymphocytes recognize major histocompatibility complex (MHC) class I receptors on muscle fibers which may cause muscle fiber damage. There is clonal proliferation of perforin-expressing CD8+ T lymphocytes within the muscle, demonstrating cytotoxicity against autologous myotubules.

Nonimmune Mechanisms

Hypoxia may be involved in DM pathogenesis. It is hypothesized that muscle fatigue in DM is related to local tissue hypoxia, a result of upregulation of vascular endothelial growth factor (VEGF) in muscle tissue. Muscle weakness improves with exercise, which is related to reduced levels of energy substrates, including ATP and phosphocreatine, indicating an acquired metabolic disturbance in these inflammatory myopathies (Grundtman et al. 2008).

MHC class 1 molecules play a critical role in initiating and perpetuating antigen-specific immune responses, by presenting antigenic peptides to CD8+ T lymphocytes and regulating the activities of natural killer (NK) cells. However, MHC class 1 cells also have nonimmune functions involved with synaptic connections of neurons and signal transduction in different cell types (Oliveira et al. 2004). They are generally not expressed, or expressed in low levels, on skeletal muscle cells but can be readily induced by proinflammatory cytokines. Activation of nuclear factor (NF)-kB subsequently activates several target genes including MHC class 1. Mouse models of myositis show that MHC class 1 molecules potentially mediate muscle fiber damage through innate (classical immune) and nonimmune (endoplasmic reticulum stress) mechanisms in the absence of lymphocytes (Nagaraju and Lundberg 2011).

Effector molecules, cytokines, and chemokines, produced by muscle fibers, are thought to contribute to the pathogenesis of myositis. Many proinflammatory cytokines are found in muscle tissue of patients with DM, namely, IL-1 alpha, TNF alpha, type 1 interferon, and HMGB1 protein. IL-1 alpha and HGMB1 are consistently found in inflammatory cells, endothelial cells, and muscle fibers in patients with persistent muscle weakness (Nagaraju and Lundberg 2011). The type I interferon system (including interferon alpha, interferon beta) has been thought to propagate autoimmune diseases by breaking tolerance and has been observed in systemic lupus erythematosus. Similarly, there seems to be a similar mechanism in the muscle and peripheral blood in DM. Type 1 interferons

are strongly associated with anti-Jo-1 and anti-SSA antibodies and correlate with MHC class 1 expression in muscle fibers (Eloranta et al. 2007). HMGB1 is a ubiquitous nonhistone molecule present in all nucleated cells and co-localizes with MHC class 1 cells. In pathogenic muscle fibers, HMGB1 is overexpressed compared to MHC class 1 cells, and in this tissue, there is an irreversible decrease in calcium release leading to a decreased effect of muscle contractility.

Finally, apoptosis of the cells are not seen in muscle specimens of myositis. Instead, necrosis and muscle fiber degeneration are evident histopathologically. Some studies have shown that antiapoptotic molecules are expressed in muscle tissue and mediate other forms of cell death, including endoplasmic reticulum (ER) response and autophagy. The ER stress response is found in patients with myositis due to an overexpression of MHC class 1 molecules in myofibers. Increased expression of ER chaperones, involved in the folding, exporting, and processing of new proteins, may be associated with further muscle fiber damage (Nagaraju and Lundberg 2011).

The pathophysiology of dermatomyositis is complex, and there are multiple interconnected immune and nonimmune mechanisms that work synergistically within the muscle and skin. Further studies are needed to fully explain muscle weakness and damage in DM and other autoimmune myositis conditions.

Workup

Clinical suspicion of DM is confirmed through a variety of tests, including biochemical markers, electromyography, and muscle biopsy. Similar findings are found in both the adult-onset and juvenile forms (Peloro et al. 2001). Creatine kinase (CK) is believed to be the most sensitive and specific marker; it can be elevated up to 50 times normal in active disease (Dalakas and Hohlfeld 2003). In amyopathic DM, however, the CK levels may be normal (Gerami et al. 2007). Other markers may be elevated in DM, including erythrocyte sedimentation rate, aldolase, lactate dehydrogenase, and aspartate/alanine aminotransferases. Elevation of these enzymes may precede clinical evidence of myositis (Mammen 2010).

Findings on EMG confirm destructive myopathy, including spontaneous activity in the form of fibrillation potentials and positive sharp waves, complex repetitive discharges, and polyphasic, short duration, small amplitude motor-unit potentials (Dalakas and Hohlfeld 2003; Bohan and Peter 1975b part 2).

Muscle biopsy in DM shows perifascicular distribution of atrophic, degenerating, and regenerating muscle fibers. A decrease in the number of capillaries is seen, though this is nonspecific, as it is also found in PM (Mammen 2010). Also seen are perivascular and *inter*fascicular inflammatory infiltrates, unlike PM in which one can see *intra*fascicular infiltrates. These lymphocytic infiltrates are primarily B cells with a smaller number of what may be CD4+ cells, though a recent study suggests they might be plasmacytoid dendritic cells (Greenberg 2008).

Skin biopsy typically shows a mild inflammatory infiltrate at the dermal-epidermal junction with vacuolar changes. It may also show perivascular inflammation of T helper cells and membrane attack complex in the dermis. It should be noted that these skin findings are not specific and may be identical in systemic lupus erythematosus (SLE). Increase in dermal mucin may be a diagnostic feature in DM compared to SLE (Smith et al. 2009). Muscle biopsy can distinguish between the two (Mammen 2010).

Myositis-specific autoantibody assays are not routinely performed for diagnostic purposes, as they occur in less than 30 % of DM patients. However, for their prognostic value, these antibodies are indeed often checked during the workup, as they can be associated with various systemic findings that may accompany DM. For instance, anti-Jo-1, associated with antisynthetase syndrome, is a commonly tested autoantibody. Anti-Mi-2 is found in 25 % of DM patients, is specific but not sensitive, and

tends to carry a more favorable prognosis with a decreased incidence of malignancy and a better response to steroid therapy (Targoff 2006). Anti-polymyositis-Scl is associated with the overlap of DM and scleroderma, and Anti-KL6 is associated with interstitial lung disease. Anti-155/140, though only found in 13–21 % of patients with DM, is highly specific for the disease and is associated with a higher rate of malignancy and lower rates of interstitial lung disease (Targoff 2006). The antibody was detected in 23 % of patients with juvenile dermatomyositis.

The association between dermatomyositis and malignancy is well known (Bohan and Peter 1975a part 1; Callen 2001). The risk of having an underlying cancer is significantly higher with DM than with PM (Hill et al. 2001). Studies confirm that there is also a high association of cancer and DM with the amyopathic form as well, though it is possible that the risk is slightly smaller compared to myopathic DM (el-Azhary and Pakzad 2002). Cancer is typically found within 1 year after the DM diagnosis, though many patients had been diagnosed with cancer several years prior to developing DM symptoms, suggesting that there might be a paraneoplastic component to the disease (Mammen 2010). Adenocarcinomas make up the majority of the malignancies diagnosed in association with DM, though there is a broad range of different types. Ovarian cancer is common but may be overrepresented in the literature (Callen 2000). Asian patients with DM commonly have nasopharyngeal cancer (Callen 2002). Most cancers tend to be discovered because of abnormal histories, physical findings, or routine laboratory testing, suggesting that doing a more extensive malignancy evaluation may not be fruitful (Callen 2002). Other studies suggest further imaging, including CT scans and ultrasounds, are in fact justified (Hill et al. 2001; Sparsa et al. 2002). Interestingly, one study showed that elevated CA-125 levels at the time of DM diagnosis was associated with an increased risk of developing cancer over the following 5 years, and this may prove to be a useful biomarker to check when performing a malignancy screen (Amoura et al. 2005).

Treatment and Management

The goals of therapy for DM aim first and foremost to improve muscle weakness and ameliorate skin disease and other systemic symptoms. Further management aims to improve quality of life, as well as routinely evaluate for associated malignancies. Generally, following the patient clinically in terms of muscle strength and dermatological improvement is the best way to determine if a particular treatment is working. Muscle enzymes can be used as a marker of improvement in muscle strength, but this does not always correlate (Miller et al. 1992) and can lead to a problem of "chasing" lab values instead of appropriately treating the patient (Dalakas and Hohlfeld 2003).

First-line treatment is corticosteroids, usually prednisone (Iorizzo and Jorizzo 2008). A typical regimen includes 80–100 mg per day for several weeks followed by a slow taper over 2–3 months. Though many patients respond well to steroids (more so in DM than PM generally), some patients require other medications for various reasons including ineffectiveness of the steroids, inability to taper the steroids without relapse of symptoms, and side-effect complications. Common, and sometimes debilitating, side effects include weight gain, acne, osteoporosis, susceptibility to infection, muscle weakness (steroid myopathy), and glucose intolerance. Prior to starting steroid treatment, one should check for tuberculosis and consider adding medications to prevent *Pneumocystis carinii* pneumonia and osteoporosis (Greenberg 2008).

Second-line treatments include a variety of immunosuppressive medications. Methotrexate is commonly used first among the immunosuppressive agents, often in conjunction with corticosteroids, in particularly severe cases of myositis. Liver toxicity is a concern, and patients with diabetes mellitus should be closely monitored (Iorizzo and Jorizzo 2008). Other immunosuppressants have been used with similar efficacy to methotrexate, though side-effect profiles may limit their long-term use. These include mycophenolate mofetil, azathioprine, cyclosporine, and cyclophosphamide (especially for

interstitial lung disease). Newer treatments such as rapamycin (Nadiminti and Arbiser 2005), anti-TNF agents such as infliximab (Iorizzo and Jorizzo 2008), and leflunomide (Boswell and Costner 2008) have been reported in case studies. A recent Cochrane review summarized IVIG to be the only efficacious systemic immunomodulator compared to placebo after 3 months for refractory DM (Dalakas et al. 1993; Gordon et al. 2012). Case reports have also demonstrated utility in patients with esophageal manifestations (Marie et al. 2010) as well as safety and efficacy when using IVIG to treat a pregnant patient suffering from DM (Mosca et al. 2005). Plasmapheresis, on the other hand, does not appear to be effective (Miller et al. 1992).

When muscle weakness is minimal and skin findings predominate, a different treatment algorithm may be pursued. Simple measures such as sun avoidance and sunscreen help prevent worsening of photosensitive rashes. Though most patients will need systemic therapy to control skin manifestations of DM, topical treatment consisting of moisturizers, antipruritics, corticosteroids, and tacrolimus/pimecrolimus can be tried first (Sontheimer 2004). First-line systemic therapy for dermatologic manifestations consists of antihistamines and 4-aminoquinoline antimalarials (hydroxychloroquine, quinacrine, chloroquine) (Woo et al. 1984). Use of hydroxychloroquine may be limited by a side effect of pruritic cutaneous eruptions (Pelle and Callen 2002). For refractory cases, systemic corticosteroids can be tried, followed by immunosuppressants and IVIG (Sontheimer 2004).

Screening for systemic involvement includes barium swallow, esophageal motility studies, chest X-ray, pulmonary function tests, and electrocardiography. Treatment of gastrointestinal and cardiopulmonary symptoms should be undertaken when appropriate. Physical therapy to prevent joint contractures should not be forgotten. A thorough history and routine examinations for malignancy screening should be done yearly for at least 3 years after diagnosis, with careful follow-up thereafter (Callen 2000, 2002; Sontheimer 2004).

Prognosis

Overall, the prognosis of DM is good. The mortality rate is somewhat unclear due to diagnostic difficulties and methodological limitations in prior studies, but a broad range of 8.9–52 % has been cited, with a lower mortality rate of 0–1.5 % in the juvenile form (Iorizzo and Jorizzo 2008). Certain characteristics portending a poorer prognosis include older age, severe muscle weakness, malignancy, cardiopulmonary involvement, and calcinosis (Marie et al. 2001). One study showed that interstitial lung disease in DM is particularly refractory to steroids, carries a poorer prognosis, and should be treated early with intensive immunosuppressive therapy. The use of corticosteroids or immunosuppressive therapies improves the prognosis (Callen and Wortmann 2006; Iorizzo and Jorizzo 2008).

Cross-References

- ▶ Autoinflammatory Diseases
- ▶ Cancer and Joint Pain
- ▶ Dermatomyositis, Skin
- ▶ Juvenile Dermatomyositis
- ▶ Juvenile Idiopathic Arthritis
- ▶ Myositis, Pathogenesis
- ▶ Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Amoura Z, Duhaut P, Huong DL, et al. Tumor antigen markers for the detection of solid cancers in inflammatory myopathies. Cancer Epidemiol Biomarkers Prev. 2005;14:1279–82.

Bohan A, Peter JB. Polymyositis and dermatomyositis (part 1). N Engl J Med. 1975a;292(7):344–7.

Bohan A, Peter JB. Polymyositis and dermatomyositis (part 2). N Engl J Med. 1975b;292(8):403–7.

Boswell JS, Costner MI. Leflunomide as adjuvant treatment of dermatomyositis. J Am Acad Dermatol. 2008;58(3):403–6.

Bradshaw EM, Orihuela A, McArdel SL, Salajegheh M, Amato AA, Hafler DA, Greenberg SA, O'Connor KC. A local antigen-driven humoral response is present in the inflammatory myopathies. J Immunol. 2007; 178(1):547–56.

Callen JP. Dermatomyositis. Lancet. 2000;355(9197): 53–7.

Callen JP. Relation between dermatomyositis and polymyositis and cancer. Lancet. 2001;357(9250):85–6.

Callen JP. When and how should the patient with dermatomyositis or amyopathic dermatomyositis be assessed for possible cancer? Arch Dermatol. 2002;138(7):969–71.

Callen JP, Wortmann RL. Dermatomyositis. Clin Dermatol. 2006;24(5):363–73.

Dalakas MC, Hohlfeld R. Polymyositis and dermatomyositis. Lancet. 2003;362:971–82.

Dalakas MC, Illa I, Dambrosia JM, Soueidan SA, Stein DP, Otero C, et al. A controlled trial of high-dose intravenous immune globulin infusions as treatment for dermatomyositis. N Engl J Med. 1993;329 (27):1993–2000.

El-Azhary RA, Pakzad SY. Amyopathic dermatomyositis: retrospective review of 37 cases. J Am Acad Dermatol. 2002;46(4):560–5.

Eloranta ML, Barbasso Helmers S, Ulfgren AK, Rönnblom L, Alm GV, Lundberg IE. A possible mechanism for endogenous activation of the type I interferon system in myositis patients with anti-Jo-1 or anti-Ro 52/anti-Ro 60 autoantibodies. Arthritis Rheum. 2007;56(9):3112–24.

Euwer RL, Sontheimer RD. Amyopathic dermatomyositis (dermatomyositis siné myositis). Presentation of six new cases and review of the literature. J Am Acad Dermatol. 1991;24(6 Pt 1):959–66. Review.

Gerami P, Schope JM, McDonald L, Walling HW, Sontheimer RD. A systematic review of adult-onset clinically amyopathic dermatomyositis (dermatomyositis siné myositis): a missing link within the spectrum of the idiopathic inflammatory myopathies. J Am Acad Dermatol. 2006;54(4):597–613.

Gerami P, Walling HW, Lewis J, Doughty L, Sontheimer RD. A systematic review of juvenile-onset clinically amyopathic dermatomyositis. Br J Dermatol. 2007;157(4):637–44. Epub 2007 Jun 26.

Gordon PA, Winer JB, Hoogendijk JE, Choy EH. Immunosuppressant and immunomodulatory treatment for dermatomyositis and polymyositis. Cochrane Database Syst Rev. 2012;8.

Greenberg SA. Inflammatory myopathies: evaluation and management. Semin Neurol. 2008;28(2):241–9.

Grundtman C, Tham E, Ulfgren AK, Lundberg IE. Vascular endothelial growth factor is highly expressed in muscle tissue of patients with polymyositis and patients with dermatomyositis. Arthritis Rheum. 2008;58(10):3224–38.

Hiketa T, Matsumoto Y, Ohashi M, Sasaki R. Juvenile dermatomyositis: a statistical study of 114 patients with dermatomyositis. J Dermatol. 1992;19(8):470–6.

Hill CL, Zhang Y, Sigurgeirsson B, et al. Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. Lancet. 2001;357(9250):96–100.

Iorizzo 3rd LJ, Jorizzo JL. The treatment and prognosis of dermatomyositis: an updated review. J Am Acad Dermatol. 2008;59(1):99–112.

Kissel JT, Mendell JR, Rammohan KW. Microvascular deposition of complement membrane attack complex in dermatomyositis. N Engl J Med. 1986;314(6): 329–34.

Levine SM, Raben N, Xie D, Askin FB, Tuder R, Mullins M, Rosen A, Casciola-Rosen LA. Novel conformation of histidyl-transfer RNA synthetase in the lung: the target tissue in Jo-1 autoantibody-associated myositis. Arthritis Rheum. 2007;56(8):2729–39.

Magro CM, Iwenofu OH, Kerns MJ, Nuovo GJ, Dyrsen ME, Segal JP. Fulminant and accelerated presentation of dermatomyositis in two previously healthy young adult males: a potential role for endotheliotropic viral infection. J Cutan Pathol. 2009;36(8):853–8.

Mammen AL. Dermatomyositis and polymyositis: clinical presentation, autoantibodies, and pathogenesis. Ann N Y Acad Sci. 2010;1184:134–53.

Marie I, Hachulla E, Hatron PY, Hellot MF, Levesque H, Devulder B, Courtois H. Polymyositis and dermatomyositis: short term and longterm outcome, and predictive factors of prognosis. J Rheumatol. 2001;28(10):2230–7.

Marie I, Menard JF, Hatron PY, Hachulla E, Mouthon L, Tiev K, et al. Intravenous immunoglobulins for steroid-refractory esophageal involvement related to polymyositis and dermatomyositis: a series of 73 patients. Arthritis Care Res (Hoboken).. 2010;62(12): 1748–55.

Miller FW, Leitman SF, Cronin ME, et al. A randomized double- blind controlled trial of plasma exchange and leukapheresis in patients with polymyositis and dermatomyositis. N Engl J Med. 1992;326:1380–4.

Mosca M, Strigini F, Carmignani A, et al. Pregnant patient with dermatomyositis successfully treated with intravenous immunoglobulin therapy. Arthritis Rheum. 2005;53(1):119–21.

Nadiminti U, Arbiser JL. Rapamycin (sirolimus) as a steroid-sparing agent in dermatomyositis. J Am Acad Dermatol. 2005;52(2 Suppl 1):17–9.

Nagaraju K, Lundberg IE. Polymyositis and dermatomyositis: pathophysiology. Rheum Dis Clin North Am. 2011;37(2):159–71.

Oliveira AL, Thams S, Lidman O, Piehl F, Hökfelt T, Kärre K, Lindå H, Cullheim S. A role for MHC class I molecules in synaptic plasticity and regeneration of neurons after axotomy. Proc Natl Acad Sci USA. 2004;101(51):17843–8.

Pelle MT, Callen JP. Adverse cutaneous reactions to hydroxychloroquine are more common in patients with dermatomyositis than in patients with cutaneous lupus erythematosus. Arch Dermatol. 2002;138(9): 1231–3. discussion 1233.

Peloro TM, Miller 3rd OF, Hahn TF, Newman ED. Juvenile dermatomyositis: a retrospective review of a 30-year experience. J Am Acad Dermatol. 2001;45(1):28–34.
Smith ES, Hallman JR, DeLuca AM, Goldenberg G, Jorizzo JL, Sangueza OP. Dermatomyositis: a clinicopathological study of 40 patients. Am J Dermatopathol. 2009;31(1):61–7.
Sontheimer RD. The management of dermatomyositis: current treatment options. Expert Opin Pharmacother. 2004;5(5):1083–99.
Sparsa A, Liozon E, Herrmann F, Ly K, Lebrun V, Soria P, Loustaud-Ratti V, Bouyssou-Gauthier ML, Boulinguez S, Bédane C, Jauberteau MO, Vidal E, Bonnetblanc JM. Routine vs extensive malignancy search for adult dermatomyositis and polymyositis: a study of 40 patients. Arch Dermatol. 2002;138(7):885–90.
Targoff IN. Myositis specific autoantibodies. Curr Rheumatol Rep. 2006;8:196–203.
Werth VP, Callen JP, Ang G, Sullivan KE. Associations of tumor necrosis factor alpha and HLA polymorphisms with adult dermatomyositis: implications for a unique pathogenesis. J Invest Dermatol. 2002;119(3):617–20.
Woo TY, Callen JP, Voorhees JJ, et al. Cutaneous lesions of dermatomyositis are improved by hydroxychloroquine. J Am Acad Dermatol. 1984;10(4):592–600.

Cancer and Fever

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Synonym

Fever in cancer; Neoplastic fever

Definition

A significant fever in patients with cancer is always a serious concern. Though it usually indicates the presence of an infection, a source is sometimes difficult to detect – and is not always found – even after an extensive investigation. This often leaves the caring physician perplexed and concerned. As cancer patients undergo several types of treatments such as antineoplastic chemotherapy, immunotherapy, targeted therapy, radiation therapy, surgery, and blood transfusions, the differential diagnosis for fever can become more complicated and requires a carefully measured evaluation. Among the variety of causes of fever, one of not uncommon etiology is fever of paraneoplastic manifestation, which is a febrile condition occurring as a result of the biologic effects from cancer itself.

Fever that occurs as a paraneoplastic manifestation is seen frequently in Hodgkin's disease and non-Hodgkin lymphomas, acute and chronic leukemias, multiple myeloma, and other solid tumors such as renal cell cancer. This paraneoplastic fever, which is defined as fever caused by cancer itself, has been shown to be the cause of fever of unknown origin (FUO) in approximately 20 % of cases as shown in Table 1 (Jacoby and Swartz 1973; Vanderschueren et al. 2003).

In this entry, several aspects of paraneoplastic fever will be discussed. The aim is to examine the differential diagnosis for various causes of fever with particular emphasis on diagnostic approaches. In addition, clinical characteristics, possible pathogenesis, and management of paraneoplastic fever will be evaluated.

Pathophysiology of Fever

"Pyrogen" is termed to describe any substance that produces fever. The pathogenesis of fever in man begins with the production of endogenous pyrogens by phagocytic leukocytes in response to exogenous pyrogens (i.e., toxins from bacteria, fungi, and yeasts, including endotoxins, exotoxins, and enterotoxins, as well as drugs such as bleomycin and cisplatin). Endogenous pyrogens, which are now known as pyrogenic cytokines, are produced as the result of an infectious as well as inflammatory process in the body. These pyrogenic cytokines are specific cytokines produced upon activation of toll-like

Cancer and Fever, Table 1 Classic causes of fever of unknown origin (FUO) in the general population (Jacoby and Swartz 1973; Vanderschueren et al. 2003)

Category	Percentage of FUO population (%)	Common
Infectious diseases	30–40 %	Tuberculosis, abscesses, osteomyelitis, endocarditis, cytomegalovirus, cat scratch disease
Connective tissue disease	20–30 %	Adult Still's disease, polymyalgia rheumatica, giant cell arteritis, polyarteritis nodosa, systemic lupus erythematosus, late-onset rheumatoid arthritis
Neoplastic disorders	20–30 %	Lymphoma, leukemia, renal cell carcinoma, hepatocellular carcinoma or other tumors metastatic to the liver, primary or metastatic central nervous system tumors
Miscellaneous disorders	15–20 %	Drug fever, alcoholic cirrhosis, Crohn's disease, subacute thyroiditis, factitious fever

receptor (TLR) (Mackowiak et al. 1992), and further regulate immune, inflammatory, and hematopoietic processes.

There are several well-known pyrogenic cytokines which include interleukin (IL)-1, IL-6, tumor necrosis factor (TNF), ciliary neurotrophic factor (CNTF), and interferon-α (IFN-α) (Dinarello 1999; Shapiro et al. 1993). Fever is produced by an interaction between pyrogenic cytokines and specialized receptors on or near thermosensitive neurons in the thermoregulatory center of the anterior hypothalamus. This interaction may cause local hypothalamic production and release of prostaglandins, monoamines, and, possibly cyclic AMP, which are all thought to have a role in fever. From the anterior hypothalamus, information is transmitted through the posterior hypothalamus to the vasomotor center, which directs sympathetic-nerve fibers to constrict peripheral vessels and decrease heat dissipation (Dinarello and Wolf 1978). Some data has suggested that although up to 70 % of patients with cancer have fever, it is more often infection or localized obstruction caused by the tumor that is responsible for the fever in the majority of the cases (Browder et al. 1961).

The pathophysiology of paraneoplastic fever in patients without overt infection or obstruction is still uncertain. However, the mechanism for paraneoplastic fever does seem to be distinct from that for fever due to infection. Research has suggested the involvement of various cytokines. Indeed, cancer cells and the immune system appear to overexpress a range of cytokines such as IL-1, TNF, IL-2, IL-6, IL-12, and interferon in patients with malignancies.

Under normal circumstances, the hematopoietic and immune systems do not produce many of the known cytokines at significant levels. However, cancer cells may produce high levels of cytokines, such as IL-1 or IL-6. Patients with lymphoma have high levels of IL-6 and IL-10. Additionally serum levels of IL-6 have correlated with the presence of B-symptoms in diffuse large cell lymphoma (Seymour et al. 1995). In patients with renal cell carcinomas, increased IL-6 levels have been associated with an increased incidence of paraneoplastic fever as well as shorter survival (Blay et al. 1992; Blay et al. 1997; Kurzrock 2001).

These cytokines can go on to act as autocrine growth factors that stimulate tumor growth and also induce production of other cytokines through the immune system. It is one of the axioms of cytokine research that virtually every cytokine induces many other cytokines and a "snowball" effect occurs once these pathways are deregulated (Kurzrock 2001).

Another mechanism for paraneoplastic fever may be related to inflammation due to tumor necrosis, which some investigators have attributed to the release of TNF and other endogenous pyrogens from dead tissue (Johnson 1996). For example, bone marrow necrosis is due to malignancy in the vast majority of cases, and fever has been documented in 68 % cases of bone marrow

necrosis. Bone marrow necrosis may also cause the release of toxins and cytokines from damaged cells (Janssens et al. 2000).

Infectious fever is produced by a combination of exogenous and endogenous pyrogens that are responsible for prostaglandin-mediated activation of thermoregulatory neurons of the anterior hypothalamic area while paraneoplastic fever is most likely primarily mediated by IL-6 and TNF, which have direct effects to thermoregulatory neurons to elevate body temperature. These differences in pathophysiology may explain not only the different clinical features between fever of infection and fever from malignancy but also the distinctly different response of the two conditions to the naproxen test.

Cancer and Fever, Table 2 Causes of fever in patients with cancer

Causes	Examples
Neoplastic origin	Hodgkin and non-Hodgkin lymphomas, acute and chronic leukemias, multiple myeloma, solid tumors such as renal cell cancer
Infections	Bacterial, fungal, viral, and/or parasitic
Drug reaction	Amphotericin B
Chemotherapy-related	Asparaginase, bleomycin, interferons
Central nervous system metastasis	Hypothalamic involvement, meningeal carcinomatosis
Radiation-induced	Radiation pneumonitis
Endocrine disorder	Steroid-induced adrenal insufficiency or crisis
Blood transfusion reaction	

Fever in Cancer

Fever, whether it presents with or without infection, requires immediate attention in cancer patients because delayed diagnosis and treatment will result in significant morbidity and mortality. Early recognition of a life-threatening infection such as pneumonia or sepsis is crucial. Additionally, the understanding of the cause of a fever and its clinical manifestations will provide timely evaluation and treatment, as well as reduce patient discomfort and unnecessary expenses. The various conditions that can cause fever in patients with cancer are summarized in Table 2.

Neutropenic Fever

In patients with cancer, especially in an immunocompromised and marrow-suppressed state, the most common cause of fever is infection. Infections may be of bacterial, viral, fungal, or parasitic origin. Fever can sometimes be the only manifestation of infection in cancer patients, particularly in those who are neutropenic, because signs and symptoms of inflammation are typically attenuated.

Fever occurs frequently during chemotherapy-induced neutropenia: 10–50 % of patients with solid tumors and >80 % of those with hematologic malignancies will develop fever during one more cycles of chemotherapy associated with neutropenia (Klastersky 2004). For most of these patients, no infectious etiology is documented. Clinically documented infections occur in 20–30 % of febrile episodes, with the intestinal tract, lung, and skin being common sites of tissue-based infection (Freifeld et al. 2011).

Most experts consider high-risk patients to be those with anticipated prolonged (>7 days duration) and profound neutropenia (absolute neutrophil count $\leq$100 cells/mm^3 following cytotoxic chemotherapy) and/or significant medical comorbid conditions, including hypotension, pneumonia, new-onset abdominal pain, or neurologic changes (Freifeld et al. 2011). During the initial assessment, a detailed history should include elicitation of new site-specific symptoms, infection exposures, and prior documented infections. Physical examination of febrile neutropenic patients requires a thorough search to detect subtle signs and symptoms. Close attention to sites which are most commonly infected is warranted. These sites include skin (including sites of procedures or central lines), oropharynx, lungs, and perineum. Laboratory tests should include a complete blood count (CBC) with differential, comprehensive metabolic panel, and at least two sets of blood cultures. In addition,

if clinically indicated, experts also recommend culture specimens from other sites of infection and a chest X-ray.

Patients with cancer and febrile neutropenia after require hospitalization for empiric IV antibiotic therapy. Monotherapy with an anti-pseudomonal B-lactam agent, such as cefepime, is initially recommended. Other antimicrobials may be added to the regimen for management of complications or if antimicrobial resistance is suspected or documented.

Drug Reaction

Another common cause of fever in cancer patients is fever caused by a reaction to drugs. Drugs utilized in the care of patients with cancer fall into two major categories – those used for supportive care and those given to treat the cancer itself (i.e., chemotherapy).

Drugs that are known to cause fever include growth factors such as filgrastim and sargramostim. Allopurinol is an uncommon but important cause of drug fever, as it is frequently used in patients with leukemia and lymphoma to prevent or diminish tumor lysis syndrome. Allopurinol-induced drug fever is often accompanied by hepatotoxicity, deterioration of renal function, severe rash, and eosinophilia (Arellano and Sacristan 1993). Amphotericin B, a parenteral agent often used in cancer patients for broad antifungal coverage, is commonly known to cause fevers, chills, and malaise 15 min to 3 h after infusion. Withdrawal of the offending drug usually results in defervescence within 72–96 h, which helps to confirm the diagnosis, but delays of 5–7 days have been observed.

The most common example of fever as an extension of the pharmacologic effect of the drug is the fever observed following chemotherapy for various solid tumors, lymphomas, and leukemias. Cell necrosis and lysis release various pyrogenic substances from damaged cells. The resulting inflammatory response is also accompanied by cytokine activation of the febrile response. Fever commences 2–3 days after chemotherapy and may last for 1 week or more. This early febrile response usually can be distinguished from febrile neutropenia which rarely develops before the 2nd week after chemotherapy.

Fever can also occur within hours after chemotherapy administration, most likely via other mechanisms. For example, a link between cytosine arabinoside (Ara-C) and fever was described in 1972, but the first systematic description was not until 1981 by Castleberry et al., who coined the term "Ara-C syndrome." Four patients with non-Hodgkin lymphoma and two with acute lymphocytic leukemia (ages 4 and 4 months to 16 years and 6 months) exhibited a unique reaction to intravenously administered Ara-C given alone as a part of the previously reported LSA2-L2 treatment protocol. The syndrome was characterized by fever, myalgia, bone pain, and occasionally by chest pain, maculopapular rash, and conjunctivitis (Castleberry et al. 1981).

More recently, a larger retrospective review was performed of 169 courses of high-dose Ara-C treatment (HDAC) administered to 57 pediatric patients. Fever occurred during 113 of the 169 HDAC courses. The fever began an average of 26 h after the start of the first infusion, with the average peak temperature 39.1 °C. In 12 of the 169 courses, an antibiotic was administered because of suspected sepsis during HDAC. All 12 of these patients had negative blood cultures, and the antibiotics were stopped after 1–3 days without relapse of fever (Ek et al. 2005). Similarly, fever after vincristine administration has also been described (Ishii et al. 1988). Interestingly, all patients were in the maintenance phases of therapy, making apoptotic tumor cells an unlikely source.

The exact mechanism of these fevers is unknown. It was previously demonstrated that pro-inflammatory cytokines such as TNF-α, IL-6, and IFN-γ are released during HDAC and probably mediate the reaction, acting as endogenous pyrogens (Ek et al. 2001). In the case of vincristine-associated fever, Kaufmann and colleagues have speculated that in patients treated with large doses of vincristine, the mechanism of fever may occur due to direct hypothalamic stimulation. However, in both of the above reports, hypersensitivity reactions could not be

ruled out, as symptoms were prevented with corticosteroids.

Hypersensitivity is in fact the most common cause of drug fever (Tabor 1986). Despite a large number of available antineoplastic agents, hypersensitivity reactions are not common except with platinum compounds, epipodophyllotoxins (etoposide), asparaginase, taxanes, and procarbazine (Shepherd 2003). Various mechanisms can cause drug fever, including the formation of circulating antibody-antigen complexes and/or a T cell immune response provoked by a drug or its metabolites. Any one episode may involve multiple antigenic determinants and mechanisms. Fever may be the sole manifestation of a hypersensitivity reaction.

As demonstrated, in most cases of fever associated with antineoplastic agents, the fever is self-limiting and can be prevented or alleviated with premedication. However, in rare cases, hyperpyrexia associated with high mortality can occur. Review of the literature reveals reported cases of fatal hyperpyrexia with bleomycin both in patients receiving the drug for the first time and also in patients who had received previous bleomycin therapy (Carter et al. 1983; Leung et al. 1989; Ma and Isbister 1980). In these cases, patients developed severe rigors and chills, with temperatures rising to as high as 42.5 °C.

Blood Transfusion

Cancer patients may develop a fever as a reaction to receiving a blood transfusion. The most common transfusion reaction is a febrile, nonhemolytic transfusion reaction (FNHTR). The clinical manifestations of this reaction include fever, chills, and sometimes mild dyspnea within 1–6 h after transfusion of red cells or platelets. FNHTRs are benign, causing no lasting sequelae, but are uncomfortable and sometimes frightening to the patient. Furthermore, since fever, with or without a chill, also may be the sign of a severe, acute hemolytic transfusion reaction or infection, FNHTRs cannot be ignored.

The management of FNHTRs should include stopping the transfusion and determining whether or not a hemolytic reaction is taking place as well as administration of antipyretics or meperidine for moderate chills and rigors. Leukoreduction of blood products before transfusion can lessen the reaction (Sirchia et al. 1987). The possibility of fever due to receiving blood products can also be lessened by giving patients acetaminophen or antihistamines before the transfusion.

Paraneoplastic Fever

When an infectious etiology of fever is not detected and noninfectious causes of fever are excluded after careful clinical examination, extensive laboratory, and imaging studies, then paraneoplastic fever should be suspected. It is estimated that paraneoplastic origin is the cause of fever in approximately 10–20 % of both immunocompetent patients and cancer patients.

Fever can be a common presentation with many malignancies. Hodgkin's disease has classically been associated with fever. However, acute leukemia, non-Hodgkin's lymphoma, renal cell carcinoma, bone sarcoma, adrenal carcinoma, neuroblastoma, and pheochromocytoma are also associated with paraneoplastic fever (Pizzo et al. 1982). Solid tumors of the breasts, lungs, and colon do not usually cause paraneoplastic fever, but the presence of liver metastases from these tumors may result in fever (Dalal and Zhukovsky 2006). In addition, any solid tumor causing obstruction can result in fever.

Definition of Paraneoplastic Fever

No clinical features reliably differentiate paraneoplastic fever from fever due to infection, fever associated with collagen-vascular disease, or fever due to other causes. Thus, paraneoplastic fever is often a diagnosis of exclusion, established after a rigorous and thorough work-up of other possible causes of fever in patients with cancer, as discussed above.

Fever due to infection is often characterized by tachycardia, hypotension, and severe chills or rigors. Shock may occur with gram-negative bacteremia. Patients are often in a very toxic state. Proportional tachycardia in response to the degree of fever occurs owing to increased basal metabolic rate and circulatory disturbances.

In contrast, patients with paraneoplastic fever tend to have persistently remittent fevers for long periods of time without much toxic effects though temperatures can be elevated above 40 °C. Chills and tachycardia are infrequent and mild, if present. The usual symptoms are excessive sweating and feelings of warmth. Almost always, paraneoplastic fever is intermittent throughout the day and night. The fever may be short lived or long lasting (Chang 1989). Paraneoplastic fever may persist for more than several months even though complete or partial remission of the cancer is achieved (Chang and Gross 1985).

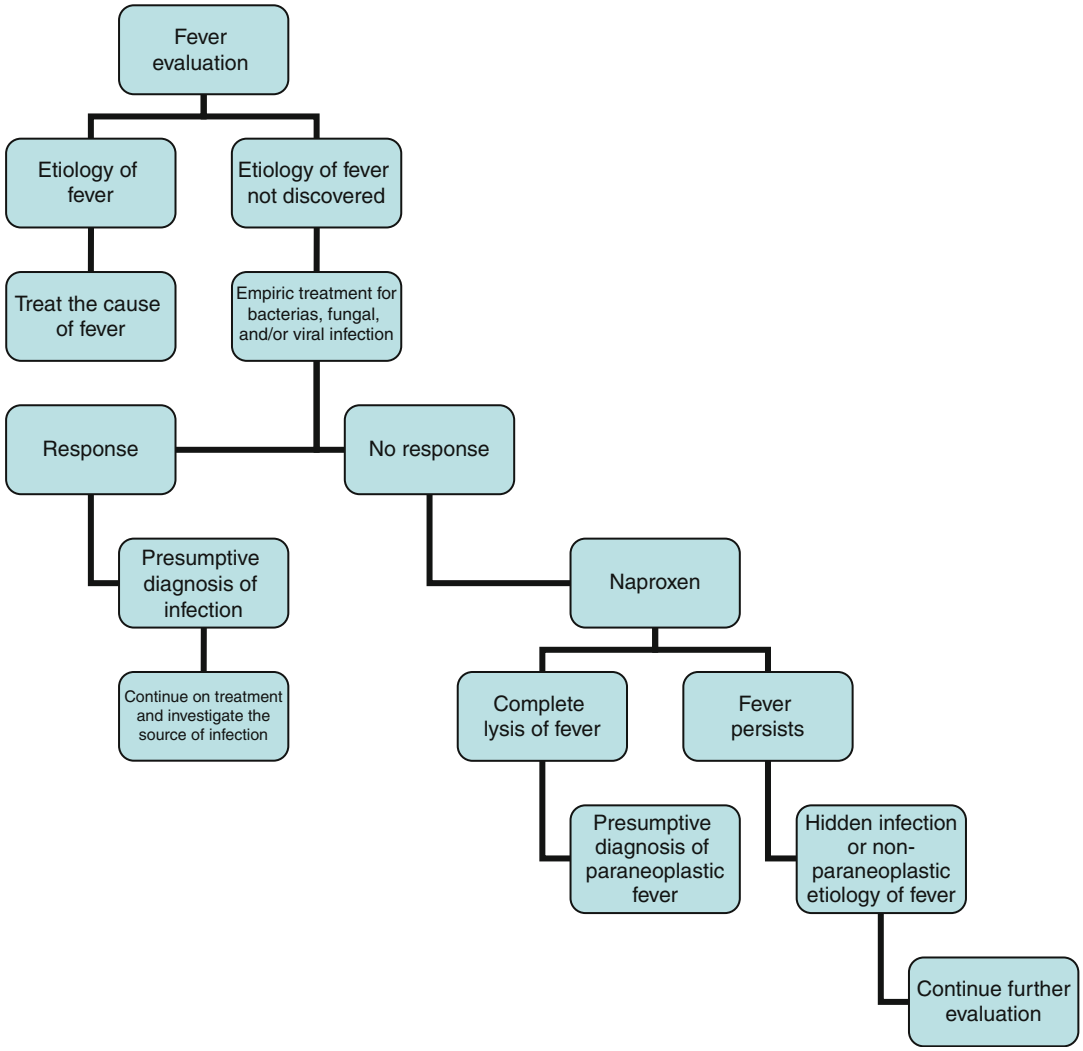
Undoubtedly, establishing the diagnosis of paraneoplastic fever on the basis of clinical findings is difficult. In addition, it should be emphasized that the degree of fever is not a distinguishing feature between infectious and paraneoplastic fevers. Objective methods, other than extensive studies to exclude infections and other causes of fever, have been introduced to help differentiate between fever due to infection and fever due to paraneoplastic fever. The nitroblue tetrazolium test was used to differentiate between fever due to bacterial infection and that due to nonbacterial infection (Feigin et al. 1971; Park et al. 1968). However, this test was later determined to be of uncertain value. In one particular study, the nitroblue tetrazolium test (NBT) showed positive reactions in all of 22 patients with Hodgkin disease and in 9 of 12 patients with malignant lymphoma. No correlation was noted in the degree of NBT reduction, activity of the diseases, presence of fever, leukocytosis, stage of the disease, or treatment modality (Chang et al. 1974).

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are commonly used nonspecific markers of inflammation. These tests have also been investigated as potential markers for differentiating fever due to an infection from paraneoplastic fever. Kallio et al. studied CRP and ESR levels in 66 hospitalized patients (56 with fever due to infection and 10 with paraneoplastic fever) on admission and compared these levels to follow-up CRP levels on hospital day 5. It was determined that CRP and ESR levels at admission were not clinically useful in differentiating paraneoplastic fever from fever due to infection. Interestingly, follow-up CRP levels were significantly lower in patients with fever due to infection when compared with those for paraneoplastic fever (Kallio et al. 2001). More recently, a systematic review of 90 studies summarized the published evidence for an association between circulating concentrations of C-reactive protein (CRP) and cancer. In most studies, CRP concentrations were found to be higher in patients with cancer than in healthy controls or controls with benign conditions. Of the nine large prospective studies identified in this entry, four reported no relationship between circulating CRP levels and breast, prostate, or colorectal cancers, and five studies found that CRP was associated with colorectal or lung cancers. However, the authors of the entry determined that most of the studies evaluating CRP as a diagnostic marker of cancer did not present relevant statistical analyses and provided no strong evidence for a causal role of CRP in cancer (Heikkila et al. 2007).

Establishing the Diagnosis of Paraneoplastic Fever

For the initial diagnosis of a fever secondary to paraneoplastic cause in a patient with fever of unknown origin, important aspects of the workup should include the careful review of clinical histories of malignancies. Attention should be paid to the presence or absence of night sweats, pruritus after a hot bath or shower, and weight loss – particularly when accompanied by a dramatic decrease in appetite. A thorough physical examination should include evaluation for abnormalities of the cranial nerves, the eyes, the throat, heart murmur, adenopathy, hepatosplenomegaly, sternal tenderness, and bone tenderness (Cunha 2007).

Ascertaining the diagnosis of paraneoplastic fever in patients with an established diagnosis of cancer should also include a careful clinical history and physical examination. In addition, the appropriate laboratory studies and imaging studies should be ordered. After these initial diagnostic steps, the following decision tree is recommended (Fig. 1).



Cancer and Fever, Fig. 1 Proposal for the diagnosis of paraneoplastic fever in patients with cancer (Adapted with permission from Chang 1989)

While awaiting laboratory results, empiric treatment with standard broad-spectrum antibacterial agents, combined with or without antifungal agents and vancomycin in certain circumstances, is warranted for at least 7 days. During this time, the clinical response and the febrile response should be followed closely. If the patient has an improved clinical response, the antibiotic therapy should be continued, even if a source of infection has not been identified (Zell and Chang 2005). If no resolution of fever occurs after empiric antibiotics and there are no contraindications (i.e., severe thrombocytopenia or allergy to nonsteroidal anti-inflammatory medications), the naproxen test can be initiated with 375 mg orally every 12 h, for at least a 36-h period (Chang 1989). The complete resolution of fever indicates a positive response to the naproxen test, which would establish a presumptive diagnosis of paraneoplastic fever. A summary of proposed criteria for establishing the diagnosis of paraneoplastic fever is shown in Table 3 (Zell and Chang 2005).

Cancer and Fever, Table 3 Proposed criteria for neoplastic fever (Adapted with permission from Zell and Chang 2005)

I. Temperature >37.8 °C at least once daily
II. Duration of fever over 2 weeks
III. Lack of evidence of infection on the following:
A. Physical examination
B. Laboratory examinations, e.g., cultures from blood, sputum, urine, stool, spinal fluid, pleural fluid, bone marrow, and discharge from local lesions
C. Radiologic examinations, e.g., chest radiographs, computerized tomography of the head, chest, abdomen, and pelvis
IV. Absence of allergic mechanisms such as drug allergy (including chemotherapy), transfusion reaction, and radiation
V. Lack of response of fever to an empiric, adequate trial of antibiotic therapy for at least 7 days
VI. Prompt and complete lysis of fever after the naproxen test, with sustained normal temperature while receiving naproxen

The Naproxen Test

The naproxen test was first described by Chang and Gross in 1984 as a reliable method in assisting in the differential diagnosis of infectious fever and paraneoplastic fever in patients with cancer and fever of undetermined origin. Twenty-two patients with cancer and fever of undetermined origin for more than 7 days were treated with naproxen 250 mg twice daily to control fever when there was no evidence of infection after a careful evaluation. Moreover, in most of these cases, antibiotic therapy had failed to resolve fevers. In this report, 14 of 15 patients with paraneoplastic fever had a complete, sustained lysis of fever while being treated with naproxen. None of five patients with infectious fever had responses to naproxen. In those patients with paraneoplastic fever, lysis was complete within 24 h, and the afebrile state continued as long as the patients were maintained on naproxen (Chang and Gross 1984).

Other nonsteroidal anti-inflammatory drugs such as indomethacin, ibuprofen, and diclofenac have also been shown to be useful in treating paraneoplastic fever. In a randomized trial of naproxen, indomethacin, or diclofenac used to ameliorate cancer-induced fever, all three drugs were equally effective in bringing the temperature down to normal. Naproxen had the most rapid effect (Lusch et al. 1968; Tsavaris et al. 1990).

Corticosteroids have been shown to cause suppression of fever caused by various etiologies, including allergic reactions, collagen-vascular diseases, and malignancy. The antipyretic effect of corticosteroids was compared to naproxen for treating paraneoplastic fever (Chang 1988). In a retrospective study of 39 patients with advanced cancer and established diagnosis of paraneoplastic fever, treatment with naproxen led to defervescence in 36 patients. Twelve of these patients also received corticosteroids at another time; all had previously responded with complete lysis of fever to naproxen. Corticosteroids induced complete lysis in only six of these patients. Though the sample size was small, these observations suggest that naproxen is more effective than corticosteroids as an antipyretic agent in the management of paraneoplastic fever.

Utility of the Naproxen Test

Follow-up data on the efficacy of the naproxen test included a total of 68 cancer patients with FUO. Statistical analysis of these data provides insight into the value of the naproxen test. In the previously mentioned report, the prevalence of paraneoplastic fever was 74 % (50 of 68 patients). The other patients described included those with infectious fever, autoimmune disease-related fever, and radiation-related fever. In the group of patients with paraneoplastic fever, 46 had complete responses, two had partial responses, and two patients had no response to naproxen. Of the 13 patients with infectious fever, all but one had no response to naproxen (Chang 1987).

Based on this data, the sensitivity and specificity of the naproxen test has previously been calculated: sensitivity is 92 % (95 % CI, 80–97 %), specificity is 100 % (95 % CI, 78–100 %), the positive predictive value is 100 % (95 % CI, 90–100 %), and the negative predictive value is 82 % (95 % CI, 59–94 %). Therefore, in a patient with a high clinical suspicion of paraneoplastic fever, the naproxen test

is highly predictive of true paraneoplastic fever (Zell and Chang 2005).

Given the high specificity of the test, if naproxen administration fails to result in complete defervescence of the patient, other etiologies need to be sought. It is still possible that a hidden or masked infection, for example, may be present. A meticulous work-up should be continued.

As an example, a 63-year-old Filipino male was undergoing treatment for acute myeloid leukemia at the local academic institution. Prior to induction with standard idarubicin and cytarabine chemotherapy, he was afebrile. However, on day #16 status-post chemotherapy, when pancytopenia had developed, the patient began to have daily fevers, as high as 39.3 °C. An extensive work-up for an infectious etiology included blood, urine, and sputum cultures, as well as computed tomography (CT) imaging of the chest, abdomen, and pelvis. Broad-spectrum antibiotics were started immediately. And yet, fevers persisted. A bone marrow biopsy performed on day #25 showed no evidence of leukemic cells. At that time, the patient was given naproxen 375 mg q12 h for 3 days. However, fevers continued, which prompted further work-up, including a lumbar puncture and transesophageal echocardiogram – neither of which revealed an infectious source. He felt clinically well, aside from poor appetite.

He did have notable findings of rising liver function enzymes. Finally, approximately 1 month after his daily fevers began, the patient underwent laparoscopic exploratory laparotomy. The surgeons noted white nodules on the gallbladder and spleen and biopsied several sites. One week later, these biopsies grew mycobacterium in culture. He had defervescence soon after treatment was initiated with ritampin, isoniazid, pyrazinamide, ethambutol (RIPE) and clarithromycin (Fig. 2).

In this complicated case, the patient's fevers could easily have been attributed to his leukemia. However, the negative naproxen test prompted the treating physicians to continue a painstaking search for a cause of fevers, ultimately requiring an invasive procedure for diagnosis. Without the naproxen test, the patient might have gone on to receive consolidation chemotherapy, putting him at great risk in the setting of serious occult infection.

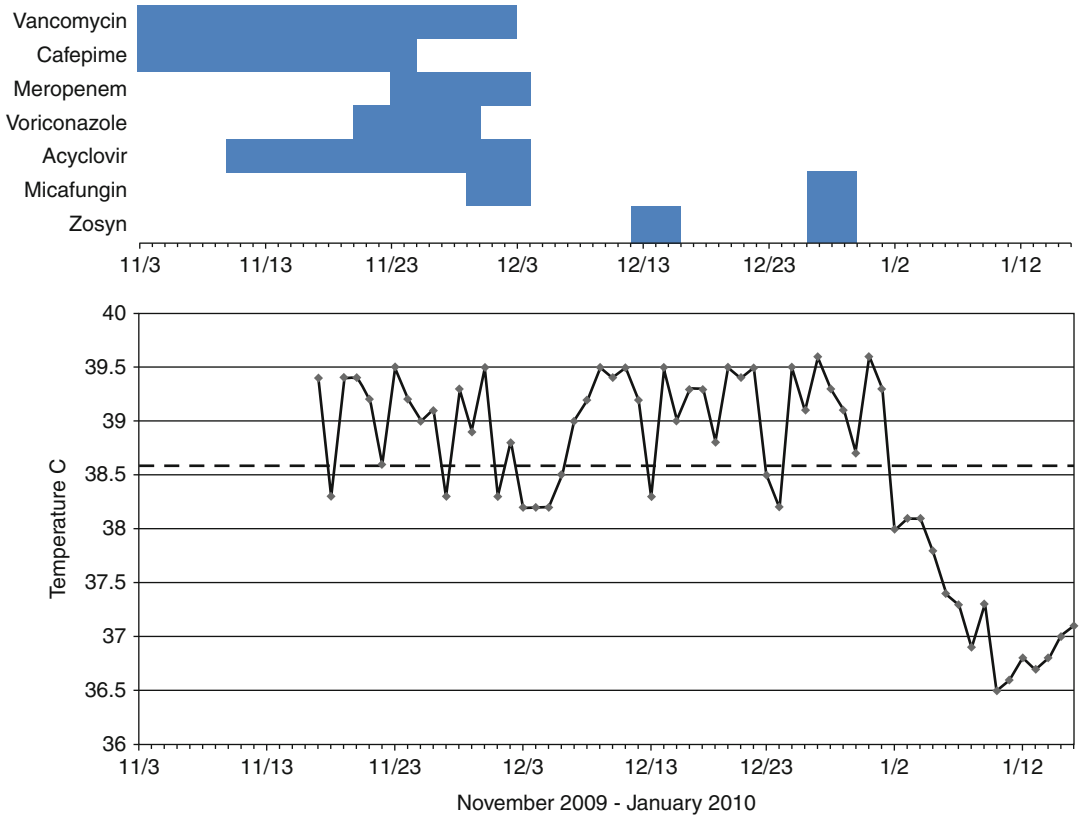
The usefulness of the naproxen test has been confirmed by others in patients with paraneoplastic fever associated with gynecologic malignancies. In one study, naproxen (250 mg orally every 8 h) was given to 12 patients with daily fevers for at least 3 days and negative work-up for infection. Within 24 h of starting naproxen therapy, 10 patients' fever responded, with subjective improvement in patient malaise and fatigue. Two patients did not respond to naproxen therapy in 24 h; thus, it was stopped and the fever work-up was continued. Of these two patients, one was eventually diagnosed with bacteremia after multiple negative blood cultures and initially no response to antibiotics (Economos et al. 1995).

Palliating Paraneoplastic Fever

Fever in patients with cancer can be extremely troubling. It is often associated with fatigue. In addition, as illustrated in the above case, fever with an unknown source demands thorough clinical examinations and a myriad of diagnostic tests which do not always carry a benign risk. Disease-specific chemotherapy may alleviate paraneoplastic fever, if the tumor is responsive to the treatment.

These findings indicate that nonsteroidal anti-inflammatory drugs can provide safe and effective palliation for distressful paraneoplastic fever as well. If naproxen is used, clinicians need to be mindful of weighing the symptomatic benefits against possible side effects such as gastritis and gastrointestinal bleeding. In addition, naproxen is to be used with caution in patients with thrombocytopenia. Other relative contraindications for naproxen use may include cardiac, renal, and hepatic dysfunction.

In some patients, paraneoplastic fever will recur if naproxen is discontinued after a short-term treatment. In a study of recurrent fever in patients with paraneoplastic fever, though naproxen induced sustained fever lysis in some



Cancer and Fever, Fig. 2 Febrile course of a 63-year-old male with acute myelogenous leukemia. Following induction chemotherapy, the patient as expected became pancytopenic but also developed unexplained fever for more than 1 month. The naproxen test on December 1 did not result in resolution of fever, prompting further work-up. After diagnosis of tuberculosis, treatment was initiated on December 29 (Figure courtesy of M. Bryan Shieh)

patients, the fever returned to pretreatment levels in a small subset of patients after naproxen withdrawal. This recurrence typically happened within 24 h after withdrawal. A detailed history taking may reveal the patient has not been compliant with naproxen. Retreatment typically results in complete and sustained fever lysis. However, if the fever is not resolved, reevaluation for infection and other causes is necessary.

Conclusions

Patients with cancer often have complicated medical courses. They can be plagued by symptoms and side effects not only from the primary malignancy but from the cancer treatment itself – whether the modality is antineoplastic, immunologic, targeted, or radiation therapy. If fever occurs, the differential diagnosis will be broad and the treating physician must work carefully through the complexities of the case to come to a solution. Especially in the case of febrile patients with cancer, establishing the correct etiology to fever in a timely manner is paramount to reducing morbidity and mortality.

As the majority of fever in cancer patients is related to infection, thorough clinical evaluations and microbiologic studies should be able to provide the diagnosis; appropriate antibiotics would resolve the fever. However, some patients do not

have responses to antibiotics, and an infectious cause cannot be identified. In these situations, it will be crucial to be able to differentiate infectious fever from paraneoplastic fever.

In difficult circumstances, the naproxen test is an additional useful agent when working through the differential diagnosis of a febrile cancer patient. This inexpensive and relatively safe medication produces complete lysis of paraneoplastic fever. It can be both a valuable diagnostic and therapeutic tool for physicians confronting fever of unknown origin.

Cross-References

- Cancer and the Central Nervous System
- Chemokines
- Interleukin-6
- Paraneoplastic Neurological Syndromes, Overview

References

Arellano F, Sacristan JA. Allopurinol hypersensitivity syndrome: a review. Ann Pharmacother. 1993;27(3):337–43.

Blay JY, Negrier S, Combaret V, Attali S, Goillot E, Merrouche Y, Mercatello A, Ravault A, Tourani JM, Moskovtchenko JF, et al. Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma. Cancer Res. 1992;52(12):3317–22.

Blay JY, Rossi JF, Wijdenes J, Menetrier-Caux C, Schemann S, Négrier S, Philip T, Favrot M. Role of interleukin-6 in the paraneoplastic inflammatory syndrome associated with renal-cell carcinoma. Int J Cancer. 1997;72(3):424–30.

Browder AA, Huff JW, Petersdorf RG. The significance of fever in neoplastic disease. Ann Intern Med. 1961;55:932–42.

Carter JJ, McLaughlin ML, Bern MM. Bleomycin-induced fatal hyperpyrexia. Am J Med. 1983;74(3):523–5.

Castleberry RP, Crist WM, Holbrook T, Malluh A, Gaddy D. The cytosine arabinoside (Ara-C) syndrome. Med Pediatr Oncol. 1981;9(3):257–64.

Chang JC. How to differentiate neoplastic fever from infectious fever in patients with cancer: usefulness of the naproxen test. Heart Lung. 1987;16:122–7.

Chang JC. Antipyretic effect of naproxen and corticosteroids on neoplastic fever. Pain Symptom Manage. 1988;3(3):141–4.

Chang JC. Neoplastic fever. A proposal for diagnosis. Arch Intern Med. 1989;149(8):1728–30.

Chang JC, Grass HM. Utility of naproxen in the differential diagnosis of fever of undetermined origin in patients with cancer. Am J Med. 1984;76(4):597–603.

Chang JC, Gross HM. Neoplastic fever responds to the treatment of an adequate dose of naproxen. J Clin Oncol. 1985;3(4):552–8.

Chang JC, Appleby J, Bennett JM. Nitroblue tetrazolium test in Hodgkin disease and other malignant lymphomas. Arch Intern Med. 1974;133(3):401–3.

Cunha BA. Fever of unknown origin: clinical overview of classic and current concepts. Infect Dis Clin North Am. 2007;21(4):867–915. vii.

Dalal S, Zhukovsky DS. Pathophysiology and management of fever. J Support Oncol. 2006;4(1):9–16.

Dinarello CA. Cytokines as endogenous pyrogens. J Infect Dis. 1999;179(Suppl 2):S294.

Dinarello CA, Wolff SM. Pathogenesis of fever in man. N Engl J Med. 1978;298:607–12.

Economos K, Lucci 3rd JA, Richardson B, Yazigi R, Miller DS. The effect of naproxen on fever in patients with advanced gynecologic malignancies. Gynecol Oncol. 1995;56(2):250–4.

Ek T, Jarfelt M, Mellander L, Abrahamsson J. Proinflammatory cytokines mediate the systemic inflammatory response associated with high-dose cytarabine treatment in children. Med Pediatr Oncol. 2001;37(5):459–64.

Ek T, Pinkava M, Abrahamsson J. Ara-C fever and infections after high-dose ara-C treatment in pediatric lymphoid malignancies. J Pediatr Hematol Oncol. 2005;27(7):364–9.

Feigin RD, Shackelford PG, Choi SC, Flake KK, Franklin Jr FA, Eisenberg CS. Nitroblue tetrazolium dye test as an aid in the differential diagnosis of febrile disorders. J Pediatr. 1971;78(2):230–7.

Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, Raad II, Rolston KV, Young JA, Wingard JR. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the infectious diseases Society of America. Clin Infect Dis. 2011;52(4):e56–93.

Heikkilä K, Ebrahim S, Lawlor DA. A systematic review of the association between circulating concentrations of C reactive protein and cancer. J Epidemiol Community Health. 2007;61(9):824–33.

Ishii E, Hara T, Mizuno Y, Ueda K. Vincristine-induced fever in children with leukemia and lymphoma. Cancer. 1988;61(4):660–2.

Jacoby GA, Swartz MN. Fever of undetermined origin. N Engl J Med. 1973;289:1407–10.

Janssens AM, Offner FC, Van Hove WZ. Bone marrow necrosis. Cancer. 2000;88(8):1769–80.

Johnson M. Neoplastic fever. Palliat Med. 1996;10(3):217–24.

Kallio R, Bloigu A, Surcel HM, Syrjälä H. C-reactive protein and erythrocyte sedimentation rate in

differential diagnosis between infections and neoplastic fever in patients with solid tumours and lymphomas. Support Care Cancer. 2001;9(2): 124–8.
Klastersky J. Management of fever in neutropenic patients with different risks of complications. Clin Infect Dis. 2004;39(Suppl 1):S32–7.
Kurzrock R. The role of cytokines in cancer-related fatigue. Cancer. 2001;92(6 Suppl):1684–8.
Leung WH, Lau JY, Chan TK, Kumana CR. Fulminant hyperpyrexia induced by bleomycin. Postgrad Med J. 1989;65(764):417–9.
Lusch CJ, Serpick AA, Slater L. Antipyretic effect of indomethacin in patients with malignancy. Cancer. 1968;21(4):781–6.
Ma DD, Isbister JP. Cytotoxic-induced fulminant hyperpyrexia. Cancer. 1980;45(9):2249–51.
Mackowiak PA, Wasserman SS, Levine MM. A critical appraisal of 98.6 degrees F, the upper limit of the normal body temperature, and other legacies of Carl Reinhold August Wunderlich. JAMA. 1992;268(12):1578.
Park BH, Fikrig SM, Smithwick EM. Infection and nitroblue-tetrazolium reduction by neutrophils. A diagnostic acid. Lancet. 1968;2(7567):532–4.
Pizzo PA, Robichaud KJ, Wesley R, Commers JR. Fever in the pediatric and young adult patient with cancer. A prospective study of 1001 episodes. Medicine (Baltimore). 1982;61(3):153–65.
Seymour JF, Talpaz M, Cabanillas F, Wetzler M, Kurzrock R. Serum interleukin-6 levels correlate with prognosis in diffuse large-cell lymphoma. J Clin Oncol. 1995;13(3):575–82.
Shapiro L, Zhang XX, Rupp RG, Wolff SM, Dinarello CA. Ciliary neurotrophic factor is an endogenous pyrogen. Proc Natl Acad Sci USA. 1993;90(18):8614.
Shepherd GM. Hypersensitivity reactions to chemotherapeutic drugs. Clin Rev Allergy Immunol. 2003;24(3):253–62.
Sirchia G, Rebulla P, Parravicini A, Carnelli V, Gianotti GA, Bertolini F. Leukocyte depletion of red cell units at the bedside by transfusion through a new filter. Transfusion. 1987;27(5):402.
Tabor PA. Drug-induced fever. Drug Intell Clin Pharm. 1986;20(6):413–20.
Tsavaris N, Zinelis A, Karabelis A, Beldecos D, Bacojanis C, Milonacis N, Karvounis N, Kosmidis P. A randomized trial of the effect of three non-steroid anti-inflammatory agents in ameliorating cancer-induced fever. J Intern Med. 1990;228(5):451–5.
Vanderschueren S, Knockaert D, Adriaenssens T, Demey W, Durnez A, Blockmans D, Bobbaers H. From prolonged febrile illness to fever of unknown origin: the challenge continues. Arch Intern Med. 2003;163(9):1033–41.
Zell JA, Chang JC. Neoplastic fever: a neglected paraneoplastic syndrome. Support Care Cancer. 2005;13(11):870–7. Epub 2005 Apr 29.

Cancer and Joint Pain

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Definition

Musculoskeletal disorders associated with malignancy may be classified as due to (A) direct tumor involvement of bones, joints, and muscles; (B) paraneoplastic syndromes; (C) altered immune surveillance; and (D) adverse reactions to anticancer therapy. Paraneoplastic rheumatic disorders are those cancer-associated rheumatic syndromes that occur at a distance from the primary tumor or metastases, and induced by the malignancy through hormones, peptides, autocrine and paracrine mediators, antibodies, and cytotoxic lymphocytes. Paraneoplastic rheumatic disorders may precede the diagnosis of the underlying tumor, occur at the same time as the tumor becomes manifest, or arise when metastases develop. In contrast to paraneoplastic syndromes, malignant transformation in the course of certain rheumatic disorders is the result of immune dysregulation; the time interval between the onset of the rheumatic disorder and diagnosis of the secondary malignancy may be as long as 20 years (Naschitz 2001; Andras et al. 2006).

Diversity of Clinical Syndromes

An expanding array of rheumatic disorders is associated with cancer (Naschitz 2001; Andras et al. 2006; Naschitz and Rosner 2008), ranging from the well-established connections with hypertrophic osteoarthropathy, cancer polyarthritis, dermatomyositis, and palmar fasciitis with polyarthritis to various more recently described associations (Table 1). Clinically, it may be difficult to distinguish paraneoplastic rheumatic disorders from direct involvement of the articular and periarticular structures by tumor.

Cancer and Joint Pain, Table 1 The spectrum of paraneoplastic rheumatic disorders[a]

Arthropathies
Local articular involvement by cancer
Rheumatoid arthritis
Cancer polyarthritis
Hypertrophic arthropathy
Polymyalgia rheumatica and atypical polymyalgia rheumatica
Palmar fasciitis and arthritis
Gout
Relapsing polychondritis
Remitting seronegative symmetrical synovitis with pitting edema
Sacroiliitis
Adult-onset Still's disease
Muscular disorders
Dermatomyositis, polymyositis, and dermatomyositis sine myositis
Localized nodular myositis
Necrotizing myopathy
Lambert-Eaton myasthenic syndrome
Scleroderma, panniculitis, and fasciitis
Systemic sclerosis
Eosinophilic fasciitis and fasciitis-panniculitis syndrome
Erythema nodosum
Panniculitis-arthritis
Vasculitides
Miscellaneous rheumatic syndromes
Reflex sympathetic dystrophy
Sjogren's syndrome
Osteomalacia
Skeletal hyperostosis
Antiphospholipid antibody syndrome
Cryoglobulinemia
Erythromelalgia

[a]Adopted from Naschitz (Naschitz 2001)

Evidence of Causality

Interpretation of causal determinism between malignancy and rheumatic disorders is affected by a number of potential sources of error. First, small series of patients are biased toward a positive association. Second, when patients, but not controls, are drawn from a hospital referral population, a "Berkson's bias" may be present, in which the possibility of referral of a patient with both a primary rheumatic disorder and malignancy is much higher than for a patient with a rheumatic disorder alone. Third, ascribing causality based only on the statistical strength of an association between two disorders, as illustrated by standardized incidence ratio (SIR) or odds ratio (OR), may be misleading, because a mere connection between two disorders does not prove causality. An association between two disorders may occur by chance or may reflect a causal relationship other than the one it suggests. For evaluation of a causal relationship between cancer and rheumatic disorders, beyond the strength of the association, additional features of the relationship need to be assessed. Sir Austin Bradford Hill proposed criteria to establish an argument of causation, and these criteria have since been widely applied (Villa et al. 2000; Hill et al. 2001).

Bradford Hill's criteria have been summarized as including (1) the demonstration of a strong association between the causative agent and the outcome; (2) consistency of the findings across research sites and methodologies; (3) the demonstration of specificity of the causative agent in terms of the outcomes it produces; (4) the demonstration of the appropriate temporal sequence, so that the causative agent occurs prior to the outcome; (5) the demonstration of a biological gradient, in which more of the causative agent leads to a poorer outcome; (6) the demonstration of a biologic rationale, such that it makes sense that the causative agent causes the outcome; (7) coherence of the findings, such that the causation argument is in agreement with what we already know; (8) experimental evidence; and (9) evidence from analogous conditions.

These nine Bradford Hill criteria give a measure of the degree in which evidence of causality between a factor and a disease may be established. However, there is no accepted scoring system of the Bradford Hill criteria. Temporal relationship is the only absolutely essential criterion, inasmuch as exposure always precedes the outcome. Clinical judgment is important when considering the guidelines together. In spite of their inherent limitations, the Bradford Hill criteria have been extensively utilized for differentiating causality from association. Two studies

applied the Bradford Hill criteria to cancer-associated musculoskeletal disorders (Naschitz and Rosner 2008; Villa et al. 2000).

Paraneoplastic Rheumatic Syndromes

In applying the Bradford Hill criteria to the association of rheumatic syndromes with cancer, Villa et al. (Villa et al. 2000) found good evidence that solid tumors are determinants of dermatomyositis (based on temporality, strength, consistency, plausibility, coherence, and analogy). The evidence that solid tumors are determinants of polymyositis was not convincing (only temporality, plausibility, coherence, and analogy criteria were satisfied).

The literature abounds in case reports and small case series describing associations between musculoskeletal disorders and cancer. However, the available database is usually insufficient for statistical analysis or judgment of causality based on Bradford Hill criteria. Based on clinical impression, but not yet supported by sufficient evidence, the following rheumatic syndromes may be paraneoplastic in nature: hypertrophic osteoarthropathy, cancer polyarthritis, palmar fasciitis and polyarthritis, and relapsing seronegative symmetric synovitis with pitting edema (Naschitz 2001; Andras et al. 2006; Guegan et al. 2006; Liozon et al. 2006).

Hypertrophic Osteoarthropathy

Hypertrophic osteoarthropathy is a prototype of rheumatic paraneoplastic syndrome (Naschitz 2001; Andras et al. 2006). The syndrome consists of clubbing of the phalanges; stiffness and swelling of joints, especially the wrists, ankles, and interphalangeal articulations; evidence of periosteal and subperiosteal new bone formation along the shaft of long bones and phalanges on radiographs; and increased vascular endothelial growth factor (VEGF) in the blood. Approximately 90 % of cases are paraneoplastic, with the remaining cases found in association with conditions such as pulmonary fibrosis, endocarditis, Graves' disease, and inflammatory bowel disease. Hypertrophic osteoarthropathy is associated with bronchial carcinoma, lung metastases, and pleural mesothelioma. Resection of the tumor is often followed by remission of the arthropathy and decrease in plasma VEGF. Other treatment options include bisphosphonates, opiate analgesics, nonsteroidal anti-inflammatory drugs, and localized palliative radiation.

Cancer Polyarthritis

Cancer polyarthritis arises predominantly in the elderly and may be oligoarticular or polyarticular, sometimes resembling adult-onset Still's disease or rheumatoid arthritis. Cancer polyarthritis differs from typical rheumatoid arthritis by generally occurring in elderly patients, having an explosive onset, being more often seronegative and asymmetric, and having no family history of rheumatoid arthritis. The lower extremities are usually involved, sometimes with sparing of the small joints of the hands and wrist. The patient does not exhibit rheumatoid nodules. Cancer polyarthritis is often refractory to conventional treatment such as NSAIDs and steroids. Lung cancer is the most common association. The onset of arthritis can precede the diagnosis of cancer by several months. Remission of the arthritis after successful treatment of the neoplasia is the post factum proof of the paraneoplastic nature of the arthritis. Only a few case series of patients with paraneoplastic arthritis have been published. A recent nationwide study from France recruited 26 patients, with a mean age 57.5 years. All had symmetrical polyarthritis involving wrists and hands (85 %). Extra-articular symptoms occurred in 84 %. There was no specific biologic or radiographic feature. The delay between the diagnoses of rheumatism and neoplasia was up to 21.2 months, with a mean of 3.6 months. Tumors were usually diagnosed after articular symptoms occurred (88.5 %). Twenty patients had a solid cancer, usually pulmonary adenocarcinoma, and six had a hematological malignancy. The tumors were diagnosed at an early stage, which may explain the relative long median survival of 1.21 years, with a mean follow-up of 1.9 years. The percentage of resolution of the articular symptoms is higher in patients with solid tumors, as compared to patients with hematological malignancy. In cases of tumor relapse, the rheumatic symptoms did not recur (sic) in 75 % of patients (Morel et al. 2008).

Palmar Fasciitis and Polyarthritis Syndrome

Palmar fasciitis ranges from diffuse globular swelling with warmth and erythema to Dupuytren's contracture. Rheumatoid factor and antinuclear antibodies are negative. This syndrome was described in association with carcinoma of the ovary, endometrium, stomach, breast, prostate, chronic lymphocytic leukemia, and Hodgkin's disease. The shoulders, metacarpophalangeal, and proximal interphalangeal joints are involved. Magnetic resonance imaging and biopsy of palmar nodules reveal inflammation and fibrosis. Anti-inflammatory treatment is usually ineffective. The rheumatic syndromes may precede tumor diagnosis by months. The tumors are often rapidly progressive. Improvement in palmar fasciitis and inflammatory arthritis often occurs following successful treatment of the ovarian carcinoma. Digital contractures, however, may persist. Gynecological examination is warranted in any woman presenting with the sudden onset of unexplained hand pain, palmar inflammatory fasciitis, palmar fibromatosis, and digital contractures (Martorell et al. 2004).

Relapsing Seronegative Symmetric Synovitis with Pitting Edema Syndrome (RS_3PE)

Relapsing seronegative symmetric synovitis with pitting edema syndrome sometimes occurs in association with malignant diseases. The symptoms appear in the form of arthritis and edema surrounding the metacarpophalangeal and interphalangeal joints and wrists. Rheumatoid factor is negative. The patients are generally elderly and male. The associated malignancy may be T cell lymphoma; myelodysplastic syndrome; colon, lung, gastric, prostate, or undifferentiated pelvis cancer; and endometrial carcinoma. In the paraneoplastic forms, symptoms typically are severe and do not respond to the established treatment. Systemic symptoms, such as fever and weight loss, often occur (Naschitz 2001; Guegan et al. 2006).

Relapsing Polychondritis

Relapsing polychondritis is a rare, chronic, inflammatory disease, with episodes of inflammation of the cartilage in the nose, ears, tracheobronchial tree, and joints. It has been reported in association with myelodysplastic syndromes, possibly as an immune response against type II collagen. There have been a few reported cases of relapsing polychondritis associated with malignant lymphoma (Yanagi et al. 2007).

Atypical Polymyalgia Rheumatica

Studies reporting cancer risk after polymyalgia rheumatica and temporal arteritis are few. Between 1965 and 2006, among 35,918 patients hospitalized for polymyalgia rheumatica and temporal arteritis, 3,941 patients developed subsequent cancer, giving an overall SIR of 1.19; for cancer diagnosed later than 1 year of follow-up, the SIR was 1.06. Hence, a marginally increased risk of cancer was noted in the first year after hospitalization (Ji et al. 2010). Clinicians should consider the possibility of underlying cancer in certain instances of polymyalgia rheumatica, although prospective studies do not suggest a general excess incidence of malignancy (Naschitz 2001; Andras et al. 2006). Atypical features such as young age, asymmetrical symptoms, relatively low erythrocyte sedimentation rate, and poor response to corticosteroid treatment should raise the suspicion of bone metastases as the cause of the patients' symptoms that resemble polymyalgia rheumatica.

Malignant Transformation During of Rheumatic Disorders

In contrast to the limited evidence of the occasional paraneoplastic nature of rheumatic syndromes, there is adequate support for the causal determinism between chronic inflammatory disorders and subsequently occurring malignancies.

The Risk of Neoplasia in the Course of Rheumatoid Arthritis

Large population-based studies have found little evidence of an increased risk of carcinoma in rheumatoid arthritis (Ekstrom et al. 2003; Bernatsky et al. 2006). The risk of cancer of the lower gastrointestinal tract may be decreased, possibly attributable to nonsteroidal anti-inflammatory drugs. On the other hand, there is an increased risk for lymphoma occurring late in

the course of rheumatoid arthritis. The largest study supporting this association involved 76,527 patients identified through the Swedish hospitalization database between 1964 and 1999 (Ekstrom et al. 2003). Five hundred and thirty five cases of lymphoma were identified, yielding SIR 2.00. Wolfe found SIR 1.9 for lymphoma among patients with rheumatoid arthritis recruited from rheumatology practices. Using population-based linked registry data from Sweden and Denmark, Landgren found an increased risk of non-Hodgkin lymphoma in patients with rheumatoid arthritis (OR 2.7). In the analysis by Villa et al. (Villa et al. 2000), six Bradford Hill criteria supported determinism of lymphoma by rheumatoid arthritis, including strength, consistency, temporality, plausibility, coherence, and analogy.

Recent studies addressed the pathogenesis of lymphoma during the course of chronic inflammation in general and rheumatoid arthritis in particular (Bernatsky et al. 2006). Nearly all B cell non-Hodgkin lymphomas express the B cell receptor, suggesting that chronic antigen stimulation plays a central role in their emergence. Failure of the antigenic stimulus to subside, as occurs with endogenous autoantigens or with persistent infections, can increase the chance of B cell transformation. Other examples of chronic stimulation by antigens leading to lymphoma include hepatitis C, Helicobacter pylori, and Epstein-Barr virus.

Methotrexate treatment has been linked to lymphomas, often Epstein-Barr virus positive, characterized by spontaneous regression once methotrexate is withdrawn. In patients who were treated by methotrexate for rheumatoid arthritis and developed non-Hodgkin's lymphoma, remission can be observed following methotrexate withdrawal, especially in non-Hodgkin's lymphoma with latency type III Epstein-Barr virus infection. The analysis of Epstein-Barr virus infection, including the latency types, might be useful to decide the optimum therapeutic strategy (Miyazaki et al. 2007). There is also data suggesting an increased risk of malignancies in rheumatoid arthritis patients who were treated with anti-TNF antibody (Breedveld et al. 2006).

Ankylosing Spondylitis

The association between ankylosing spondylitis and malignant lymphomas was assessed in a nationwide, population-based, case–control study of 50,615 cases of lymphoma, and 92,928 matched controls, by using prospectively recorded data on lymphomas from the Swedish Cancer Register (1964–2000) and data on pre-lymphoma hospitalizations for ankylosing spondylitis from the Swedish Inpatient Register (1964–2000). There was no increased risk of lymphoma among patients with ankylosing spondylitis (Askling et al. 2006).

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is associated with a long-term risk of developing malignant lymphoma. The association between SLE and malignancy fulfilled five Bradford Hill criteria demonstrating causality (strength, temporality, plausibility, coherence, and analogy) (Villa et al. 2000).

Systemic Sclerosis

The association between systemic sclerosis and malignancy fulfilled six Bradford Hill criteria in Villa's study (strength, consistency, temporality, plausibility, coherence, and analogy); the authors concluded that there is evidence of causality between systemic sclerosis and lung cancer (Villa et al. 2000).

Sjogren's Syndrome

An increased risk of developing lymphoma has been identified in Sjogren's syndrome (Soderberg et al. 2006). The association between Sjogren's syndrome and lymphoma fulfilled six Bradford Hill criteria (strength, consistency, temporality, plausibility, coherence, and analogy); the authors concluded that there is evidence of causality between Sjogren's syndrome and lymphoma (Villa et al. 2000).

Which Work-Up for Which Patients?

Paraneoplastic musculoskeletal disorders may precede the diagnosis of cancer by months or years, thus representing a potential marker for occult malignancy (Naschitz 2001; Andras et al. 2006). Diagnosing some of those syndromes may

be challenging because certain paraneoplastic musculoskeletal disorders are rare and the clinician's opportunity to gain experience with such syndromes is limited. The prevalence of paraneoplastic rheumatic syndromes in a cohort of 3,770 patients with newly diagnosed solid tumors was 2.65 %, with arthritis and Raynaud's prevailing: both paraneoplastic syndromes were linked to malignancies of the urogenital system. The patients' immunologic status did not help differentiate between paraneoplastic and other arthritides (Rugienė et al. 2011). Further difficulty stems from the fact that paraneoplastic rheumatic syndromes may be clinically indistinguishable from disorders caused by infiltration of musculoskeletal organs by cancer cells, whether primary or metastatic. It is generally accepted that an extensive search for malignancy in most patients with recent-onset musculoskeletal disorders of unknown etiology is not cost-effective and thus not to be recommended, unless a patient presents additional findings suggestive of malignancy. Rheumatic syndromes that may be clues of occult neoplasia have been reviewed elsewhere (Naschitz 2001; Andras et al. 2006).

The question of whether "tumor markers" and markers of altered immunity may be useful in the work-up of patients with recent-onset musculoskeletal disorders has not been settled (Rugienė et al. 2011). Recent studies revealed a layer of genetic programmatic coordination by which cells determine their fate; this layer involves posttranscriptional regulation of gene expression by microRNAs. Defining the molecular taxonomy of tumors by microRNAs patterns and applying molecular taxonomy patterns to the diagnosis of neoplasia are tasks for future studies.

An increased incidence of antinuclear antibodies (ANA) has been detected in malignant conditions, but no ANA specificities (anti-ENA, anti-DNA) have been recognized in patients with malignancies. Hypocomplementemia was present in a quarter of patients with Sjogren's syndrome and correlated with lymphoproliferative disorders and mortality (Ramos-Casals 2004). Circulating monoclonal immunoglobulins were detected in one-fifth of patients with Sjogren's syndrome, most commonly monoclonal IgG (Brito-Zeron et al. 2005). Relevance of the latter immunologic finding for diagnostic purposes has not been established (Naschitz 2001; Andras et al. 2006).

Conclusions

There is firm epidemiologic evidence that cancer may present with paraneoplastic dermatomyositis, less so for polymyositis. Additionally, there is a prevailing clinical impression, but scarce epidemiological evidence, that certain musculoskeletal disorders may be paraneoplastic in nature. The role of specific clinical findings and biological markers, as hints of a possible neoplastic etiology of musculoskeletal syndromes, has not been solved. Strong evidence has accumulated on the role of longstanding rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus, and systemic sclerosis as premalignant conditions.

Cross-References

- ▶ Cancer and Dermatomyositis
- ▶ Cancer and Joint Pain
- ▶ Cutaneous Vasculitis
- ▶ Giant Cell Arteritis
- ▶ Raynaud's Phenomenon
- ▶ Scleroderma-Like Conditions of the Skin

References

Andras C, Csiki Z, Ponyi A, et al. Paraneoplastic rheumatic syndromes. Rheumatol Int. 2006;26:376–82.

Askling J, Klareskog L, Blomqvist P, et al. Risk for malignant lymphoma in ankylosing spondylitis: a nationwide Swedish case–control study. Ann Rheum Dis. 2006;65:1184–7.

Bernatsky S, Ramsey-Goldman R, Clarke A. Malignancy and autoimmunity. Curr Opin Rheumatol. 2006;18:129–34.

Breedveld FC, Weisman MH, Kavanaugh AF, et al. The PREMIER study: a multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum. 2006;54:26–37.

Brito-Zeron P, Ramos-Casals M, Nardi N, et al. Circulating monoclonal immunoglobulins in Sjogren syndrome: prevalence and clinical significance in 237 patients. Medicine. 2005;84:90–7.

Ekstrom K, Hjalgrim H, Brandt L, et al. Risk of malignant lymphomas in patients with rheumatoid arthritis and in their first-degree relatives. Arthritis Rheum. 2003;48:963–70.
Guegan S, Poszepczynska-Guigne E, Wechsler J, et al. Severe necrotic and haemorrhagic edema of the upper limbs revealing remitting seronegative symmetrical synovitis with pitting edema. Ann Dermatol Venereol. 2006;133:795–8.
Hill CL, Zhang Y, Sigurgeirsson B, et al. Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. Lancet. 2001;357:96–100.
Ji J, Liu X, Sundquist K, Sundquist J, et al. Cancer risk in patients hospitalized with polymyalgia rheumatica and giant cell arteritis: a follow-up study in Sweden. Rheumatology (Oxford). 2010;49:1158–63.
Liozon E, Loustaud V, Fauchais AL, et al. Concurrent temporal (giant cell) arteritis and malignancy: report of 20 patients with review of the literature. J Rheumatol. 2006;33:1606–14.
Martorell EA, Murray PM, Peterson JJ, et al. Palmar fasciitis and arthritis syndrome associated with metastatic ovarian carcinoma: a report of four cases. J Hand Surg [Am]. 2004;29:654–60.
Miyazaki T, Fujimaki K, Shirasugi Y, et al. Remission of lymphoma after withdrawal of methotrexate in rheumatoid arthritis: relationship with type of latent Epstein-Barr virus infection. Am J Hematol. 2007;82:1106–9.
Morel J, Deschamps V, Toussirot E, et al. Characteristics and survival of 26 patients with paraneoplastic arthritis. Ann Rheum Dis. 2008;67:244–7.
Naschitz JE. Rheumatic syndromes: clues to occult neoplasia. Curr Opin Rheumatol. 2001;13:62–6.
Naschitz JE, Rosner I. Musculoskeletal syndromes associated with malignancy. Curr Opin Rheumatol. 2008;20:100–5.
Ramos-Casals M. Hypocomplementemia in systemic lupus erythematosus and primary antiphospholipid syndrome: prevalence and clinical significance in 667 patients. Lupus. 2004;13:777–83.
Rugienė R, Dadonienė J, Aleknavičius E, et al. Prevalence of paraneoplastic rheumatic syndromes and their antibody profile among patients with solid tumours. Clin Rheumatol. 2011;30:373–80.
Soderberg KC, Jonsson F, Winqvist O, et al. Autoimmune diseases, asthma and risk of haematological malignancies: a nationwide case–control study in Sweden. Eur J Cancer. 2006;42:3028–33.
Villa AR, Kraus A, Alarcon-Segovia D. Autoimmune rheumatic diseases and cancer: evidence of causality? In: Shoenfeld Y, Gershwin ME, editors. Cancer and autoimmunity. Amsterdam: Elsevier; 2000. p. 111–7.
Yanagi T, Matsumura T, Kamekura R, et al. Relapsing polychondritis and malignant lymphoma: is polychondritis paraneoplastic? Arch Dermatol. 2007;143:89–90.

Cancer and Nephrotic Syndrome

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Synonyms

Focal segmental glomerulonephritis; IgA nephropathy; Light chain nephropathy; Membranoproliferative glomerulonephritis; Membranous glomerulonephritis; Minimal change disease; Paraneoplastic glomerulonephritis; Rapidly progressive glomerulonephritis; Renal amyloidosis

Definition and Background

Nephrotic syndrome describes the onset of heavy proteinuria (>3.0 g/24 h), hypertension, hypercholesterolemia, edema, and microscopic hematuria. The term *nephrotic range proteinuria* is used when patients demonstrate heavy proteinuria without the aforementioned clinical manifestations. Nephrotic syndrome has been described in a number of renal syndromes including but not limited to minimal change disease (MCD), focal segmental glomerulonephritis (FSGS), membranous glomerulonephritis, and renal amyloidosis (Lewis and Neilson 2012).

Nephrotic syndrome is a rare complication of both solid and hematologic malignancies that is often described as a paraneoplastic syndrome. Paraneoplastic nephropathies are not directly related to tumor burden, invasion, or metastasis, but are caused by the secretion of hormones, growth factors, cytokines, and tumor antigens by the tumor itself (Ronco 1999). The first series of paraneoplastic glomerulonephritis was published by Lee et al. in 1966 (Lee et al. 1966).

While membranous nephropathy in patients with solid tumors and minimal change disease in patients with Hodgkin's lymphoma are considered the classic glomerulonephropathies associated with malignancy, cancer-associated nephrotic syndrome encompasses a plethora of glomerulonephropathies including FSGS, membranoproliferative glomerulonephritis (MPGN), IgA nephropathy, and rapidly progressive glomerulonephritis (RPGN). Paraneoplastic glomerulonephritis improves with the remission of the underlying malignancy. Therefore, early recognition of these disorders is important as treatment differs from idiopathic glomerulonephropathies. While paraneoplastic glomerulonephritis must be considered if nephrotic range proteinuria occurs in the setting of malignancy, diagnosis is established when proteinuria remits with treatment of the malignancy and/or recurs with recurrence of the underlying malignancy (Lefaucheur et al. 2006; Bacchetta et al. 2009).

Solid Tumors

Membranous Nephropathy

Membranous nephropathy has been most consistently linked to malignancy. The largest series of patients with membranous nephropathy demonstrated a 10 % prevalence of cancer (Lefaucheur et al. 2006). Paraneoplastic membranous nephropathy is more comment in older individuals (age >50), current or former tobacco users (>20 pack-year history), and male gender (Lefaucheur et al. 2006; Ronco 1999). Membranous nephropathy has been seen most commonly in prostate adenocarcinoma and lung carcinoma; however, it has also been reported in breast carcinoma, renal cell carcinoma, gastric adenocarcinoma, colon adenocarcinoma, neuroblastoma, neuroendocrine tumors, GIST, hepatocellular carcinoma, choriocarcinoma, carcinoid tumor, and nasopharyngeal carcinoma (Becker et al. 1996; Lefaucheur et al. 2006; Wagrowska-Danilewicz and Danilewicz 2011).

On pathology, membranous nephropathy is characterized by subendothelial immune complex deposition. An autopsy series of patients with malignancy demonstrated that 17 % of kidneys contained glomerular deposits on immunofluorescence (Beaufils et al. 1985). Another series suggests that by electron microscopy, 55 % of patients with cancer have immune complex deposition consistent with membranous nephropathy (Pascal et al. 1976). While carcinoembryonic antigen, prostate-specific antigen, and melanoma antigens have been associated with membranous nephropathy, it is not clear that they are the causative agents. Ohanti et al. demonstrated that patients with paraneoplastic membranous nephropathy have significantly more IgG1 and IgG2 deposition compared to those with idiopathic membranous nephropathy. However, IgG4 was equally expressed in both paraneoplastic and idiopathic membranous nephropathy (Ohtani et al. 2004). As IgG1 and IgG2 formation is regulated by the type 1 T-helper (Th1) cell release of cytokines (i.e., IL-12 and interferon), some hypothesize that Th1-mediated responses in malignancy may drive membranous nephropathy (Ohtani et al. 2004; Lien and Lai 2011).

Minimal Change Disease (MCD)

MCD has been observed in lung, colorectal, and renal cancers. Some theorize that MCD in malignancy may be related to increased expression of vascular endothelial growth factor (VEGF). This is supported by pediatric studies that show increased VEGF expression in the podocytes of patients who have nephrotic syndromes (Ostalska-Nowicka et al. 2005). VEGF has been associated with increased membrane permeability (Taniguchi et al. 2004). Taniguchi et al. reported a case in which a patient with rectal adenocarcinoma had elevated VEGF and nephrotic syndrome at diagnosis; however, after resection of the tumor, proteinuria resolved and VEGF normalized.

Membranoproliferative Glomerulonephritis (MPGN)

MPGN has been rarely reported in lung, renal, and gastric cancers. While some cases have demonstrated improvement in nephrotic syndrome with resection of the malignancy, others have

improved with prednisone (Ahmed et al. 2008). Therefore, some hypothesize that MPGN may be associated with immune complex deposition induced by tumor antigens.

Rapidly Progressive Glomerulonephritis (RPGN)

RPGN has been reported in renal call, lung, and gastric cancers. However, many of the cases reported were prior to elucidation of anti-neutrophil cytoplasmic antibodies (ANCA). It is known that ANCA-associated vasculitides carry a higher risk for development of malignancy. Additionally, cases in which RPGN resolved with treatment of the underlying malignancy inevitably required immunosuppressive therapy, which would concomitantly treat an underlying vasculitis (Lien and Lai 2011). The correlation between RPGN and malignancy is less clear.

IgA Nephropathy

IgA nephropathy has been identified in patients with carcinomas of the respiratory tract, nasopharynx, and renal cell. There have been cases of Henoch-Schonlein purpura in lung cancers and other malignancies (Pertuiset et al. 2000; Flynn et al. 2011). Anticancer therapy has been used to treat this disorder but the pathophysiology of paraneoplastic IgA nephropathy is not well understood (Mustonen et al. 1984).

Thrombotic Microangiopathy

Thrombotic microangiopathy is characterized by thrombocytopenia, microangiopathic hemolytic anemia, neurologic alteration, and acute kidney injury. It is believed to be caused by microvascular tumor emboli. Therefore, thrombotic microangiopathy is not classified as a paraneoplastic syndrome, but often presents with nephrotic range proteinuria as well. It has been described in gastric, lung, and breast cancers and carries a poor prognosis. In the Oklahoma thrombocytopenic thrombotic microangiopathy (TTP)/hemolytic uremic syndrome (HUS) registry, 10 patients with cancer-associated thrombotic angiopathy were identified; none of these patients improved with plasma exchange, and all died after diagnosis. This demonstrates that thrombotic microangiopathy causing nephrotic syndrome carries a different pathophysiology compared to TTP.

Hematologic Malignancies

Minimal Change Disease (MCD)

MCD occurs in approximately 1 % of patients with Hodgkin's lymphoma. It has also been reported in chronic lymphocytic lymphoma (CLL), even in early stage disease (Alzamora et al. 2006), myelodysplastic disorders, and in thymomas as well, particularly lymphocyte-predominant thymomas. In the post-allogeneic transplant setting, appearance of MCD is considered to be a presentation of graft-versus-host disease (GVHD), as donor T cells have been observed to infiltrate the renal parenchyma (Romagnani et al. 2005). Immunosuppressive treatment for GVHD has also improved proteinuria in these cases (Silva et al. 2007).

In Hodgkin's lymphoma, MCD has been associated with increased inflammatory markers, particularly type 2 T-helper cell (Th2) cytokines. In a rat model, it has been shown that overexpression of IL-13 (a Th2 cytokine) results in a MCD-like nephropathy (Lai et al. 2007). It is known that IL-13 is also overexpressed in Hodgkin's disease (Ohshima et al. 2001). While there appears to be an association between IL-13 and MCD, it is not clear whether IL-13 itself directly causes increased membrane permeability that results in MCD. The relationship between Th2 response and MCD has been further demonstrated in thymomas. In lymphocyte-predominant thymomas, MCD persists even after tumor excision and improves with steroid treatment (Karras et al. 2005). Rat models of thymoma show a similar phenomenon in which MCD persists despite thymectomy. In these rats, there is increased Th2-mediated cytokines, and suppression of the Th2 axis improves the degree of proteinuria (Le Berre et al. 2005). Models in thymoma and Hodgkin's disease suggest that regulation of the Th2 axis may be helpful in the treatment of MCD.

Focal Segmental Glomerulonephritis (FSGS)

FSGS occurs in 0.1 % of patients with Hodgkin's lymphoma and in approximately 3 % of patients with myeloproliferative disorders (e.g., polycythemia vera, essential thrombocythemia). It has also been seen in myelodysplastic disorders. Patients with high platelet counts in particular have elevated platelet-derived growth factor (PDGF), which has been found to enhance mesangial proliferation and fibrosis in vitro (Gersuk et al. 1989). Supporting this hypothesis, PDGF is not elevated in chronic myelogenous leukemia that is not associated with FSGS (Floege et al. 2008).

Membranoproliferative Glomerulonephritis (MPGN)

MPGN has been seen in CLL, non-Hodgkin's lymphomas, and hairy cell leukemia. Some cases have been associated with a mixed cryoglobulinemia (types I and II) involving a monoclonal immunoglobulin. Additionally, patients with hepatitis C-associated mixed cryoglobulinemia whose proteinuria does not respond to traditional hepatitis C therapy have been found to respond to rituximab, an anti-B-cell antibody (anti-CD-20). These findings may imply that B cell proliferation has a role in the development of MPGN (Cacoub et al. 2008).

Membranous Nephropathy

Membranous nephropathy has been rarely seen in CLL, chronic myelomonocytic leukemia, and non-Hodgkin's lymphomas. When it occurs, it presents with subepithelial deposits and monoclonal IgG kappa light chain deposits, suggesting that antibodies produced by monoclonal B cells may play a role in the development of membranous nephropathy (Evans et al. 2003).

IgA Nephropathy

IgA nephropathy has been associated with cutaneous T cell lymphomas. As the Th2 cytokine profile is dominant in cutaneous T cell lymphomas, skewed immunoregulation has been implied in the development of IgA nephropathy in this disorder (Bajel et al. 2009).

Light Chain Related Nephropathy

Both multiple myeloma and amyloidosis cause nephrotic range proteinuria through a number of mechanisms. Kappa and lambda free light chains produced in both disorders are freely filtered across the glomerulus. They overwhelm the ability of the proximal tubule cells to endocytose them. Therefore, they form tubular casts with Tamm-Horsfall protein (uromodulin) that can cause tubular obstruction, which decreases the glomerular filtration rate. It is believed that the Tamm-Horsfall proteins interact with the variable regions of the light chains, which may explain why light chain burden does not correlate directly with the degree of nephropathy (Comenzo et al. 2001). Additionally, the endocytosed light chains in the renal tubule cells induce a proinflammatory response (activation of nuclear factor kappa B and release of IL-6, IL-8, and tumor necrosis factor-a), which subsequently causes deposition of matrix protein that further disrupts glomerular integrity (Sengul et al. 2002). Lastly, the deposition of immunoglobulins themselves, in the form of amyloid and non-amyloid, through every portion of the kidney independently cause nonselective proteinuria (Dimopoulos et al. 2008).

Summary

Both solid and hematologic malignancies are associated with nephrotic range proteinuria through various mechanisms. Specific glomerulonephropathies have been associated with different types of malignancies. It appears that monoclonal formation of antibodies, as well as polarized Th1 versus Th2 responses, are mediators of the development of paraneoplastic glomerulonephropathies. However, the exact pathophysiology underlying these disorders has yet to be better elucidated.

Cross-References

- ▶ IgA Nephropathy
- ▶ Paraneoplastic Neurological Syndromes, Overview

References

Ahmed MS, Wong CF, Abraham KA. Membranoproliferative glomerulonephritis associated with metastatic prostate carcinoma–should immunosuppressive therapy be considered? Nephrol Dial Transplant: Off Publ Eur Dial Transpl Assoc Eur Ren Assoc. 2008;23(2):777.

Alzamora MG, Schmidli M, Hess U, Cathomas R, von Moos R. Minimal change glomerulonephritis in chronic lymphocytic leukemia: pathophysiological and therapeutic aspects. Onkologie. 2006;29(4):153–6.

Bacchetta J, Juillard L, Cochat P, Droz JP. Paraneoplastic glomerular diseases and malignancies. Crit Rev Oncol Hematol. 2009;70(1):39–58.

Bajel A, Yin Lin M, Hill PA, Goodman D, McCormack C, Foley P, et al. IgA nephropathy associated with cutaneous T cell lymphoma. Leuk Lymphoma. 2009;50(12):2083–5.

Beaufils H, Jouanneau C, Chomette G. Kidney and cancer: results of immunofluorescence microscopy. Nephron. 1985;40(3):303–8.

Becker BN, Goldin G, Santos R, Glick A, Johnson DH, Breyer JA, et al. Carcinoid tumor and the nephrotic syndrome: a novel association between neoplasia and glomerular disease. South Med J. 1996;89(2):240–2.

Cacoub P, Delluc A, Saadoun D, Landau DA, Sene D. Anti-CD20 monoclonal antibody (rituximab) treatment for cryoglobulinemic vasculitis: where do we stand? Ann Rheum Dis. 2008;67(3):283–7.

Comenzo RL, Zhang Y, Martinez C, Osman K, Herrera GA. The tropism of organ involvement in primary systemic amyloidosis: contributions of Ig V(L) germ line gene use and clonal plasma cell burden. Blood. 2001;98(3):714–20.

Dimopoulos MA, Kastritis E, Rosinol L, Blade J, Ludwig H. Pathogenesis and treatment of renal failure in multiple myeloma. Leuk: Off J Leuk Soc Am Leuk Res Fund UK. 2008;22(8):1485–93.

Evans DJ, Macanovic M, Dunn MJ, Pusey CD. Membranous glomerulonephritis associated with follicular B-cell lymphoma and subepithelial deposition of IgG1-kappa paraprotein. Nephron Clin Pract. 2003;93(3):c112–8.

Floege J, Eitner F, Alpers CE. A new look at platelet-derived growth factor in renal disease. J Am Soc Nephrol: JASN. 2008;19(1):12–23.

Flynn AN, du Prey B, Al Ardati H, Raman M, Lemaire J. Adult-onset malignancy-associated Henoch-Schonlein purpura. Scand J Rheumatol. 2011;40(4):325–6.

Gersuk GM, Carmel R, Pattengale PK. Platelet-derived growth factor concentrations in platelet-poor plasma and urine from patients with myeloproliferative disorders. Blood. 1989;74(7):2330–4.

Karras A, de Montpreville V, Fakhouri F, Grunfeld JP, Lesavre P. Renal and thymic pathology in thymoma-associated nephropathy: report of 21 cases and review of the literature. Nephrol Dial Transplant: Off Publ Eur Dial Transpl Assoc Eur Ren Assoc. 2005;20(6):1075–82.

Lai KW, Wei CL, Tan LK, Tan PH, Chiang GS, Lee CG, et al. Overexpression of interleukin-13 induces minimal-change-like nephropathy in rats. JAm Soc Nephrol: JASN. 2007;18(5):1476–85.

Le Berre L, Herve C, Buzelin F, Usal C, Soulillou JP, Dantal J. Renal macrophage activation and Th2 polarization precedes the development of nephrotic syndrome in Buffalo/Mna rats. Kidney Int. 2005;68(5):2079–90.

Lee JC, Yamauchi H, Hopper Jr J. The association of cancer and the nephrotic syndrome. Ann Intern Med. 1966;64(1):41–51.

Lefaucheur C, Stengel B, Nochy D, Martel P, Hill GS, Jacquot C, et al. Membranous nephropathy and cancer: epidemiologic evidence and determinants of high-risk cancer association. Kidney Int. 2006;70(8):1510–7.

Lewis JB, Neilson EG. Chapter 283. Glomerular diseases. In: Longo DLFA, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, editors. Harrison's principles of internal medicine. 18th ed. New York: McGraw-Hill; 2012. p. 1782–96.

Lien YH, Lai LW. Pathogenesis, diagnosis and management of paraneoplastic glomerulonephritis. Nat Rev Nephrol. 2011;7(2):85–95.

Mustonen J, Pasternack A, Helin H. IgA mesangial nephropathy in neoplastic diseases. Contrib Nephrol. 1984;40:283–91.

Ohshima K, Akaiwa M, Umeshita R, Suzumiya J, Izuhara K, Kikuchi M. Interleukin-13 and interleukin-13 receptor in Hodgkin's disease: possible autocrine mechanism and involvement in fibrosis. Histopathology. 2001;38(4):368–75.

Ohtani H, Wakui H, Komatsuda A, Okuyama S, Masai R, Maki N, et al. Distribution of glomerular IgG subclass deposits in malignancy-associated membranous nephropathy. Nephrol Dial Transplant: Off Publ Eur Dial Transpl Assoc Eur Ren Assoc. 2004;19(3):574–9.

Ostalska-Nowicka D, Zachwieja J, Nowicki M, Kaczmarek E, Siwinska A, Witt M. Vascular endothelial growth factor (VEGF-C1)-dependent inflammatory response of podocytes in nephrotic syndrome glomerulopathies in children: an immunohistochemical approach. Histopathology. 2005;46(2):176–83.

Pascal RR, Iannaccone PM, Rollwagen FM, Harding TA, Bennett SJ. Electron microscopy and immunofluorescence of glomerular immune complex deposits in cancer patients. Cancer Res. 1976;36(1):43–7.

Pertuiset E, Liote F, Launay-Russ E, Kemiche F, Cerf-Payrastre I, Chesneau AM. Adult Henoch-Schonlein purpura associated with malignancy. Semin Arthritis Rheum. 2000;29(6):360–7.

Romagnani P, Lazzeri E, Mazzinghi B, Lasagni L, Guidi S, Bosi A, et al. Nephrotic syndrome and renal failure after allogeneic stem cell transplantation: novel molecular diagnostic tools for a challenging differential diagnosis. Am J Kidney Dis: Off J Natl Kidney Found. 2005;46(3):550–6.

Ronco PM. Paraneoplastic glomerulopathies: new insights into an old entity. Kidney Int. 1999;56(1):355–77.
Sengul S, Zwizinski C, Simon EE, Kapasi A, Singhal PC, Batuman V. Endocytosis of light chains induces cytokines through activation of NF-kappaB in human proximal tubule cells. Kidney Int. 2002;62(6):1977–88.
Silva S, Maximino J, Henrique R, Paiva A, Baldaia J, Campilho F, et al. Minimal change nephrotic syndrome after stem cell transplantation: a case report and literature review. J Med Case Reports. 2007;1:121.
Taniguchi K, Fujioka H, Torashima Y, Yamaguchi J, Izawa K, Kanematsu T. Rectal cancer with paraneoplastic nephropathy: association of vascular endothelial growth factor. Dig Surg. 2004;21(5–6):455–7.
Wagrowska-Danilewicz M, Danilewicz M. Nephrotic syndrome and neoplasia: our experience and review of the literature. Pol J Pathol: Off J Pol Soc Pathol. 2011;62(1):12–8.

Cancer and Neuropathies

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Synonyms

Immune-mediated neuropathy in cancer; Inflammatory neuropathy associated with malignancy; Paraneoplastic neuropathy; Peripheral neuropathy in cancer; Polyneuropathy in cancer

Definition

Neuropathy refers to the injury or dysfunction of the peripheral nerves. The peripheral nerves are those that are located outside of the brain and spinal cord, providing sensory, motor, and autonomic innervation to all organs and structures of the body. Depending on the cause of the neuropathy, sensory nerves, motor nerves, and/or autonomic nerve fibers may be affected to varying degrees. As a result, interruption of normal peripheral nerve function can result in pain, tingling, numbness, weakness, clumsiness, gait imbalance, and/or autonomic dysfunction, such as blood pressure fluctuations, temperature dysregulation, and gastrointestinal dysfunction.

Patients with cancer are subject to the development of peripheral neuropathy on a number of bases. Neuropathic dysfunction of the peripheral nerves in patients with cancer occurs most frequently as a side effect of chemotherapeutic agents used for treatment. Additional etiologies include direct tumor invasion of the peripheral nerves or nerve plexi, involvement of spinal nerves in leptomeningeal carcinomatosis, and collateral damage to nerve structures from radiation treatment.

In addition, several *paraneoplastic* syndromes have been identified in which peripheral neuropathy is either the presenting or the predominant characteristic. Paraneoplastic syndromes are broadly defined as a symptom constellation that occurs as a remote effect of cancer on the nervous system, mediated by an immunological process. In some cases, a specific type of neuropathy with characteristic features is associated with an identifiable antibody (*onconeural* antibody) and specific types of tumor. In the malignant plasma cell proliferative disorders, direct activity of monoclonal immunoglobulins is implicated in the pathogenesis of various neuropathies (Antoine and Camdessanche 2007; DeAngelis and Posner 2009).

Basic Characteristics

Paraneoplastic Neuropathy Associated with Onconeural Antibodies

The most widely recognized antibody associated with neuropathy occurring on a paraneoplastic basis is the *anti-Hu antibody*, also referred to as the *antineuronal nuclear antibody-1 (ANNA-1)*. First described by Denny-Brown in 1948, the anti-Hu paraneoplastic syndrome is characterized by sensory neuronopathy and/or encephalomyelitis associated with serum autoantibodies directed against antigens expressed by certain tumors, most commonly small cell lung cancer (Dalmau et al. 1992).

The classical presentation of this disorder is development of an acute-subacute pure sensory neuropathy without motor involvement. It affects women more than men, with an age of onset typically between 50 and 80 years old. Concomitant encephalomyelitis is present in about 25–50 % of cases. Neuropathic symptoms include pain and numbness, often asymmetric at onset, evolving over weeks to months to involve all extremities and occasionally the face and trunk. Sensory ataxia may be severe, affecting the ability to walk or even sit unsupported. Deep tendon reflexes are typically absent. Less commonly, anti-Hu antibodies are implicated in other neuropathy phenotypes, including mixed sensorimotor and predominantly motor neuropathies. Motor weakness of the limbs and rarely face may therefore be present, in rare cases manifesting as the prominent symptom. Dysautonomia and cranial neuropathies, though even more rare, may also occur.

The diagnosis is made by a combination of clinical signs and symptoms as well as detection of anti-Hu antibodies in either the serum or CSF. The majority of patients diagnosed with the syndrome do not have a known cancer diagnosis at the time of onset of the neurological symptoms. The time to definitive diagnosis of identifiable cancer can range from several months to several years. Small cell lung carcinoma accounts for 70–90 % of these cases, though many other cancer types have been implicated, including prostate, breast, ovarian, pancreatic, neuroendocrine, thymic, and bladder.

In addition to anti-Hu, other onconeural antibodies have been associated with specific neuropathy syndromes in patients with cancer.

Anti-CV2 onconeural antibodies have been associated with sensory and sensorimotor neuropathy in the setting of small cell lung cancer and less frequently thymoma and non-Hodgkin's lymphoma. CV2 antibodies are also associated with cerebellar ataxia and uveitis.

Ganglionic nicotinic acetylcholine receptor binding antibodies are associated with a syndrome of acute autonomic neuropathy, presenting with severe orthostatic hypotension, nausea and vomiting after meals, reduced sweating, dry eyes and mouth, sexual dysfunction, and/or bladder dysfunction. In about 20 % of cases, sensory neuropathy is also present, usually prominently affecting the small fiber nerves and manifesting as pain. As with anti-Hu and anti-CV2 neuropathies, this paraneoplastic neuropathy syndrome is also most frequently observed in patients with small cell carcinoma of the lung, although it has also been reported in patients with other malignancies, particularly thymoma (Rudnicki and Dalmau 2005).

The pathophysiology of these syndromes is believed to occur via an immune response triggered by the neoplasm in which an antibody generated against the tumor becomes misdirected toward a tissue that expresses the same (or sufficiently similar) antigen. In the case of the paraneoplastic neuropathies, the antibodies target various sites along the peripheral neurons and/or nerves, resulting in the injury and dysfunction of these structures. This theory is supported by studies showing that in patients with anti-Hu antibodies, tumors tend to be smaller and may even spontaneously regress (Dalmau et al. 1999).

Neuropathies and Plasma Cell Proliferative Disorders

Multiple myeloma, osteosclerotic myeloma, Waldenstrom's macroglobulinemia, as well as other plasma cell dyscrasias are often associated with peripheral neuropathy (as is monoclonal gammopathy of uncertain significance or MGUS). Multiple neuropathy variants occur in disorders associated with monoclonal paraproteins, including inflammatory demyelinating neuropathies, as well as those with features of predominantly axonal injury. Usually, specific autoantibodies known to target peripheral nerve antigens are not identified.

Clinical studies have demonstrated evidence of preexisting peripheral neuropathy in up to 25 % of patients with multiple myeloma prior to their treatment with potentially neurotoxic chemotherapy agents. Osteosclerotic myeloma is a rare variant of myeloma with a very high prevalence of peripheral neuropathy. Many patients with this disorder will present with symptoms of neuropathy, either

as part of the POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes) associated with this type of cancer or as an isolated feature. The incidence of peripheral neuropathy in Waldenstrom's macroglobulinemia is possibly as high as 47 %.

In the plasma cell dyscrasias resulting in monoclonal gammopathies, it is believed that in some cases the paraproteins react immunologically with specific peripheral nerve or neuron structures, in some cases acting as antibody. In patients with IgM monoclonal gammopathy and neuropathy, antibodies with reactivity directed against the myelin-associated glycoprotein (anti-MAG) may be present. *Anti-MAG* neuropathy predominantly involves sensory nerves, and prominent tremor and sensory ataxia are commonly seen features. Anti-MAG antibodies have been identified in 5–45 % of patients with Waldenstrom's macroglobulinemia. In other cases, IgM or IgG reactivity toward specific ganglioside subtypes is associated with specific phenotypes of neuropathy (Latov 1995; Ropper and Gorson 1998).

Treatment

There are two main modes of treatment for immune-mediated neuropathies associated with cancer: suppressing the immune response and treating the underlying malignancy. Immune suppression typically consists of using corticosteroids, cyclophosphamide, azathioprine, plasma exchange, and/or intravenous immunoglobulin to curb the autoantibody activity directed against the peripheral nerves. Unfortunately, immunomodulatory treatment is often unsatisfactory, leading to stability of symptoms in some patients but only very rarely improvement. Treatment of the underlying malignancy generally yields the greatest improvement. Studies have demonstrated that antitumor therapy is the most significant factor in creating stabilization and improvement in patients with anti-Hu syndrome, and in approximately two-thirds of multiple myeloma patients, the neuropathy responds to successful treatment of the cancer.

Cross-References

- ▶ Cancer and Dermatomyositis
- ▶ Cancer and the Central Nervous System
- ▶ Paraneoplastic Neurological Syndromes, Overview

References

Antoine JC, Camdessanche JP. Peripheral nervous system involvement in patients with cancer. Lancet Neurol. 2007;6:75–86.

Dalmau J, Graus F, Rosenblum MK, Posner JB. Anti-Hu-associated paraneoplastic encephalomyelitis/sensory neuronopathy. A clinical study of 71 patients. Medicine. 1992;71:59–72.

Dalmau J, Gultekin HS, Posner JB. Paraneoplastic neurologic syndromes: pathogenesis and physiopathology. Brain Pathol. 1999;9:275–84.

DeAngelis LM, Posner JB. Neurologic complications of cancer. 2nd ed. New York: Oxford University Press; 2009.

Latov N. Pathogenesis and therapy of neuropathies associated with monoclonal gammopathies. Ann Neurol. 1995;37(S1):S32–42.

Ropper AH, Gorson KC. Neuropathies associated with paraproteinemia. N Engl J Med. 1998;338:1601–7.

Rudnicki SA, Dalmau J. Paraneoplastic syndromes of the peripheral nerves. Curr Opin Neurol. 2005;18:598–603.

Cancer and the Central Nervous System

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Synonyms

Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-prionic acid; AMPA; ANNA; Antineuronal nuclear antibody; Collapsin response mediator protein; CRMP; GABA; GAD; Glutamic acid decarboxylase; Metabotropic glutamate receptor type 1; mGLuR1; NMDA; *N*-methyl-D-aspartate; PCA; Polypyrimidine-tract binding;

PTB; Purkinje cell autoantibody; Tubby-like protein 1; TULP1; VGCC; VGKC; Voltage-gated calcium channel; Voltage-gated potassium channel; γ-Aminobutyric acid-B

Definition

The central nervous system (CNS) may be effected by cancer both directly, through metastatic disease and direct tumor extension, or indirectly. Damage to the nervous system, remote from the site of tumor and not associated with toxicity or metabolic abnormalities associated with cancer or its treatment, is termed paraneoplastic neurological syndromes (PNS). PNS, which are believed to exert their effect through an immune-mediated mechanism (Darnell 1996), may affect any neuronal cell type in the peripheral or central nervous system. While PNS may present as a generalized or diffuse disorder, such as a paraneoplastic encephalomyeloneuritis, there are several recognized or classical clinical syndromes which may affect certain anatomic locations of the central nervous system. The clinical characteristics and pathophysiology of the recognized paraneoplastic syndromes of the CNS, including limbic encephalitis, paraneoplastic cerebellar degeneration, opsoclonus-myoclonus, retinopathies, and stiff person syndrome will be the focus of this entry.

Clinical Presentation and Diagnosis

PNS usually present in an acute to subacute manner, over days to weeks, producing severe disability followed by stabilization. Laboratory evaluation usually demonstrates evidence of inflammation in the cerebral spinal fluid (CSF) such as lymphocytic pleocytosis, elevated protein, and oligoclonal banding, as well as brain magnetic resonance imaging (MRI) abnormalities on fluid attenuation inversion recovery (FLAIR)/T2 sequences. In the majority of cases, the PNS will likely be the initial presentation, the underlying cancer only discovered after a recognized syndrome spurs a search for malignancy.

Key to the diagnosis of PNS is the discovery of antineuronal antibodies in the serum and CSF leading to the postulate of autoimmunity as the pathophysiological basis of PNS. When these antibodies are markers of an underlying cancer, they are termed onconeural antibodies (Musunmuru et al. 2001). Over the years, certain well-characterized onconeural antibodies have been associated with certain clinical presentations representing classic PNS as well as certain underlying malignancies (Table 1). These include anti-Hu, anti-Yo, anti-CV2, anti-Ri, anti-Ma2, and anti-amphiphysin. When found, these may help focus the search for cancer to a few organs.

Recently, these onconeural antibodies have been classified as belonging to one of two groups. Antibodies from group 1 are found to target intracellular neuronal antigens; those from group 2 target neuronal surface antigens (Graus et al. 2010). It has proven difficult to produce animal models of disease based on passive transfer of group 1 antibodies, which include the well-characterized onconeural antibodies, suggesting these antibodies are likely not directly pathogenic but may represent the humoral component of a more complex cellular response (Tanaka et al. 2004). Antibodies from group 2, which usually target synaptic cell surface membrane proteins, appear to have a more direct pathogenic role given their good response to immunotherapy, sometimes regardless of disease duration, and correlation of disease response to antibody titer (Dalmau 2011). While there is a strong association in this second group with certain characteristic CNS syndromes, a paraneoplastic syndrome is less certain as there may or may not be an associated malignancy.

Classic PNS of the CNS

While PNS may present with diffuse or multifocal encephalomyeloneuritis, there are certain distinct classical clinical presentations

Cancer and the Central Nervous System, Table 1 Paraneoplastic syndromes of the CNS and associated tumors and antibodies

Clinical syndrome	Associated tumors	Associated antibodies	References
Limbic encephalitis	SCLC, testicular cancer, thymoma, teratoma, Hodgkin's disease, non-SCLC	**Anti-Hu** (ANNA-1), **anti-Yo** (PCA-1), **anti-Ri** (ANNA-2), ANNA-3, anti-Ma1, **anti-Ma2** (Ta), **anti-amphiphysin**, **anti-CRMP5** (CV2), PCA-2, anti-CRMP3, 4, anti-VGKC, anti-NMDAR, anti-$GABA_B$, anti-AMPA	Luque (1991), Knudsen (2007), Dalmau (2007), Gultekin (2000), Lawn (2003), Tuzun (2007), Dalmau (2008), Irani (2011), Boronat (2011), Rosenfeld (2010)
Cerebellar degeneration	Breast cancer, ovarian cancer, SCLC, Hodgkin's disease	**Anti-Yo** (PCA-1), **anti-Hu** (ANNA-1), anti-Tr, **anti-Ri** (ANNA-2), anti-mGluR1, anti-VGCC, anti-Ma1, anti-**CRMP5** (CV2), anti-Zic4, anti-VKCC, anti-mGLuR1	Shams'ili (2003), Peterson (1992), Hammack (1992), Clouston (1992), Darnell (2006), Clouston (1992), Sillevis (2000)
Opsoclonus-myoclonus	Neuroblastoma, SCLC, breast	**Anti-Ri** (ANNA-2), **anti-Yo** (PCA-1), **anti-Hu**(ANNA-1), anti-Ma1, **anti-Ma2** (Ta), **anti-amphiphysin**, **anti-CRMP5** (CV2)	Wong (2007), Bataller (2001), Dranell (2006), Luque (1991)
Retinopathies	SCLC, melanoma, breast	**Anti-recoverin**, anti-enolase, anti-TULP1, anti-PTB-like protein, anti-photoreceptor cell-specific nuclear receptor, **anti-CRMP5** (CV2)	Adamus (2004), Kikuchi (2000), Tateiwa (2001), Eichen (2001), Cross (2003)
Stiff person syndrome	Breast cancer, SCLC, Hodgkin's disease	**Anti-amphiphysin**, anti-GAD, **anti-Ri** (ANNA-2), anti-gephyrin	Lockman (2007), Pittock (2005), McCabe (2004), Butler (2000)

CRMP collapsin response mediator protein, *ANNA* antineuronal nuclear antibody, *PCA* Purkinje cell autoantibody, *VGKC* voltage-gated potassium channel, *NMDA N*-methyl-D-aspartate, *GABA* γ-aminobutyric acid-B, *AMPA* alpha-amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid, *VGCC* voltage-gated calcium channel, *mGLuR1* metabotropic glutamate receptor type 1, *TULP1* tubby-like protein 1, *PTB* polypyrimidine-tract binding, *GAD* glutamic acid decarboxylase. Well-characterized onconeural antibodies are in bold

historically discussed in the context of PNS of the CNS. A number of these are discussed below.

Limbic Encephalitis

Paraneoplastic limbic encephalitis (PLE), first named 40 years ago, represents an inflammatory process affecting the mesial temporal lobes and limbic mesial cortical structures. PLE will usually present rapidly, within days to weeks. Patients typically present with memory loss, confusion, psychiatric abnormalities, and seizures. The initial personality changes and psychiatric manifestations including hallucinations, agitation, anxiety, and depression may lead to a presumptive diagnosis requiring admission to a psychiatric hospital, before the development of seizures and altered consciousness spur further evaluation.

The discovery of onconeural antibodies aids in diagnosis, although up to 10 % of patients with PLE will be seronegative or express uncharacterized antibodies (Graus et al. 2008). Eighty percent of patients have MRI T2-weighted and FLAIR hyperintensity in the bilateral mesial temporal lobes, although these areas rarely demonstrate gadolinium (GAD) enhancement. Focal or generalized slowing, and epileptiform activity, maximal in the temporal lobes, is seen on electroencephalography (EEG) in all (Lawn et al. 2003). CSF examination frequently shows

signs of inflammation with elevated protein, lymphocytic pleocytosis, and oligoclonal banding.

Small-cell lung cancer is associated with PLE in 50–60 % of patients, followed by testicular germ cell tumors (20 %) and breast cancer (8 %) (Gultekin et al. 2000). Other cancers associated with PLE include thymoma, ovarian teratoma, non-SCLC, and Hodgkin's disease (Table 1).

Limbic encephalitis (LE) has come to be characterized based on the location of target antigens, being either intracellular or neuronal cell surface receptors (Rosenfeld et al. 2010). Anti-Hu and anti-Ma2 are the most commonly discovered onconeural antibodies to intracellular antigens in LE. Anti-Hu, seen in up to 60 % of cases where antibodies are present, is the most common intracellular paraneoplastic antibody and, when positive in limbic encephalitis, denotes a 94 % correlation with underlying SCLC (Gultekin et al. 2000). Many patients with anti-Hu-associated PLE will have involvement in other areas of the nervous system, such as a subacute sensory neuronopathy (SSN). Anti-Hu antibodies, also frequently found in patients with SCLC without PLE, though in much lower titers, are associated with an intranuclear RNA-binding protein which functions in cell cycle regulation (Dalmau et al. 1990). Anti-Ma2 is a second intracellular antibody, primarily seen in young men who develop PLE in association with an underlying testicular germ cell tumor (Dalmau et al. 2004). They may have involvement outside the limbic system, including the hypothalamus, producing excessive daytime sleepiness, REM-sleep abnormalities, and hyperphagia. Treatment response is poor in these patients, as with other less frequently discovered intracellular antibodies, including anti-collapsin response mediator protein (CRMP) (CV2) and anti-amphiphysin (Tüzün et al. 2007). However, a dramatic response can be seen in anti-Ma2-associated PLE following immunotherapy and cancer treatment.

A more recently emerging discovery is limbic encephalitis associated with antibodies directed towards cell surface or synaptic proteins including the voltage-gated potassium channel (VGKC) (Vincent et al. 2004), the *N*-methyl-D-aspartate (NMDA) receptor (Irani et al. 2011), the γ-aminobutyric acid-B ($GABA_B$) (Boronat et al. 2011) receptor, and the alpha-amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid (AMPA) receptor (Bataller et al. 2010). These newly discovered cell surface receptor antibodies may account for a majority of the patients with PLE and SCLC who were previously found to be seronegative for the well-characterized intracellular antibodies (Boronat et al. 2011). Unlike the well-characterized onconeural antibodies, these newly discovered antibodies, directed towards cell surface proteins, may in some cases be associated with an idiopathic or autoimmune neurological syndrome with no underlying malignancy. In addition, the antibodies are likely themselves pathogenic, and consequently, patients with these syndromes usually improve with immunotherapy aimed at removing antibodies form serum, such as IVIg and plasma exchange, as well as with treatment of the underlying tumor.

LE associated with VGKC antibodies is found to be paraneoplastic in about 30 % of cases, commonly occurring with SCLC and thymoma (Dalmau et al. 2008). A percentage of these patients may also develop a peripheral nerve hyperexcitability syndrome, such as neuromyotonia or Morvan's syndrome (neuromyotonia with CNS symptoms of hallucinations, delusions, and insomnia). NMDA receptor-associated LE is often described, in about 60 %, as occurring in a paraneoplastic form in young women with ovarian teratoma (Irani et al. 2011). In addition to the psychiatric symptoms and seizures common in PLE, these patients also have prominent dysautonomia, with labile heart rate and blood pressure, frequent hypoventilation, as well as a characteristic movement disorder with semi-repetitive orofacial and limb movements and dystonic posturing (Dalmau et al. 2008). Early and prominent seizures are a common presentation of LE associated with antibodies to the $GABA_B$ receptor. This syndrome is associated with SCLC in about

50 % of cases. $GABA_B$ receptor antibodies may be present, with glutamic acid decarboxylase (GAD) antibodies (Boronat et al. 2011). Anti-AMPA receptor encephalitis, usually seen in middle-age women, has an approximately 70 % association with an underlying tumor of the breast, lung, or thymus (Dalmau et al. 2010).

Cerebellar Degeneration

Paraneoplastic cerebellar degeneration (PCD), the first PNS to be recognized, is the best described and well documented and one of the most common paraneoplastic presentations of cancer. Sometimes preceded by a prodromal viral-like illness, cerebellar symptoms, including truncal and appendicular ataxia, nystagmus, and dysarthria, dominate the clinical picture. PCD may begin abruptly, progress over weeks to months, and then stabilize, usually leaving the patient significantly impaired, unable to walk or sit unassisted, and unable to perform fine motor tasks such as writing or eating. The degree and probability of severe impairment correlates somewhat with the underlying cancer and type of antineuronal antibody present.

As in PLE, there is usually evidence of inflammation in the CSF, though MRI usually does not demonstrate pathology until cerebellar atrophy develops late in the disease. MRI evidence of cerebellar swelling and enhancement of the cerebellar folia are reported by some (Darnell et al. 2006). During the early stages, fluorodeoxyglucose-PET may demonstrate cerebellar hypermetabolism, followed by evidence of hypometabolism in late stages (Dalmau et al. 2008). Histological findings, in the cerebellar cortex and deep cerebellar nuclei, include extensive loss of Purkinje cells with inflammatory infiltrates present at early stages of disease (Verschuuren et al. 1996).

Malignancies commonly associated with PCD include gynecologic cancers of the breast and ovary, SCLC, and Hodgkin's disease. Though not hard and fast, a correlation exists between certain paraneoplastic antibodies occurring in PCD and certain clinical features and cancer types. Breast or gynecologic cancer is usually detected with anti-Yo positivity (Peterson et al. 1992). Present in 38 % of patients in whom antibodies are detected, anti-Yo is the most common antibody in PCD. It targets the cdr2 antigen, normally expressed on Purkinje cells in the cerebellum and aberrantly expressed in ovarian and breast cancers.

With anti-Yo PCD, cerebellar symptoms usually present in isolation, often leaving patients with significant long-term disability resulting from irreversible Purkinje cell destruction. Similar to other antibodies that target intracellular neuronal antigens, treatment response is poor, although aggressive cancer treatment and immunotherapy, if initiated early in the course of disease, may improve the possibility of success (Dalmau et al. 2008). There are a few case reports of IVIg, given at a dose of 2 g/kg divided over 2–5 days, resulting in neurological improvement in patients with PCD and detectable anti-Yo antibodies. However, this is the exception rather than the rule (Widdess-Walsh et al. 2003). Patients who improved were treated within 1 month of symptom onset and usually received concurrent cancer therapy. Treatment at 1–3 months resulted in stable disease, and treatment outside 3 months usually had a poor outcome (Widdess-Walsh et al. 2003). Anti-Hu antibodies with SCLC also herald a poor prognosis, and while the cerebellar symptoms may predominate, other symptoms coexist, suggesting a more diffuse encephalomyelitis.

Hodgkin's disease and anti-Tr antibodies are also commonly associated with PCD. While anti-Yo antibodies are found in older women with PCD, anti-Tr antibodies are more commonly present in young men, reflecting the demographics of the underlying tumor. Hodgkin's disease, in contrast to other tumors, usually precedes the diagnosis of PCD, possibly because B symptoms lead to earlier diagnosis (Hammack et al. 1992). Patients with anti-Tr antibodies have a better neurological prognosis than those with Yo or Hu antibodies.

PCD, as is the case in LE, may also occur in the setting of antibodies directed against cell

surface antigens. P/Q type voltage-gated calcium channel (VGCC) antibodies are associated with SCLC and Lambert-Eaton myasthenic syndrome (LEMS). PCD sometimes occurs in SCLC, with LEMS, and high titers of VGCC antibodies. These patients may not exhibit any other recognized anti-Purkinje cell autoantibody and may harbor VGCC antibodies without developing LEMS, suggesting a role in the pathogenesis of cerebellar dysfunction (Mason et al. 1997). However, in that study, treatment with immunotherapy, as opposed to other paraneoplastic syndromes associated with neuronal receptor antibodies, did not alter the course of PCD. There have also been reports of PCD in the setting of Hodgkin's disease and antibodies against a glutamate receptor, metabotropic glutamate receptor type 1 (mGLuR1) (Sillevis Smitt et al. 2000).

Other associated antibodies include anti-CRMP5 and anti-Zic4 with SCLC. In patients with encephalomyelitis and testicular cancer, the presence of anti-Ma1 and anti-Ma2 antibodies correlated with development of cerebellar dysfunction (Darnell 2004).

Opsoclonus-Myoclonus

Opsoclonus-myoclonus (OM) syndrome comprises myoclonic jerks of the limbs and trunk, with opsoclonus, involuntary, arrhythmic, high-amplitude, and multidirectional saccades of the eyes. Opsoclonus may be constant, even during sleep, and may cause oscillopsia or blurring and oscillation of vision. OM is often associated with cerebellar ataxia, most often in children, and commonly referred to as opsoclonus-myoclonus-ataxia syndrome, though adult forms exist. Besides the classic triad of opsoclonus, myoclonus, and ataxia, pediatric patients in particular may exhibit sleep disturbance, cognitive dysfunction, and behavioral disruption. Neurological symptoms, especially in children, may resolve spontaneously or with treatment but may relapse. Brain MRI is usually normal but a mild CSF pleocytosis may be seen.

Pediatric OM etiology is diverse, including para- and post-infectious, toxic, and paraneoplastic causes. Pediatric OM is paraneoplastic in 40 % of patients, always associated with neuroblastoma. It is the most common pediatric paraneoplastic neurological syndrome in the medical literature, although it remains quite rare, occurring in 2–3 % of pediatric neuroblastoma (Rudnick et al. 2001). As the neurological symptoms in many cases of pediatric paraneoplastic OM respond well to immunotherapy, including IVIg, corticosteroids, and adrenocorticotropic hormone (ACTH), there does not appear to be permanent neuronal degeneration. More likely, transient antibody-mediated dysfunction occurs (Wong 2007). Furthermore, despite normal CSF cell counts, there is B cell expansion, which has been proposed as a candidate biomarker for the disease (Gorman 2010). A recent study demonstrated clinical improvement and reduced CSF B cells with rituximab, an agent that targets B cells, supporting a B-cell-mediated process (Pranzatelli et al. 2006). However, it has been difficult to consistently isolate specific autoantibodies, although recently, antibodies to surface proteins in cerebellar granular neurons in OM have been reported, and these exhibit a cytotoxic effect on neuroblastoma cells (Blaes et al. 2005). This antitumor immune response may explain the favorable prognosis for survival in children with coincident OM and neuroblastoma.

Among adults, paraneoplastic OM and anti-Ri antibodies are associated, usually in women with underlying breast cancer (Pittock et al. 2003). The Ri antibody recognizes the RNA-binding protein, Nova, which is strictly neuron specific and may regulate neuronal RNA metabolism (Musunuru et al. 2010). OM is also seen in patients with SCLC but predominately without the identification of a paraneoplastic antibody. However, anti-Hu and anti-amphiphysin are documented (Bataller et al. 2001). In contrast to children, adult paraneoplastic OM does not respond well to immunotherapy. Partial or complete neurological recovery may be seen with treatment of the underlying tumor (Bataller et al. 2001).

Retinopathies

PNS affecting the visual system predominantly involve the retina and optic nerve. Three distinct paraneoplastic visual syndromes are recognized: cancer-associated retinopathy (CAR), melanoma-associated retinopathy (MAR), and paraneoplastic optic neuropathy (PON) (Damek 2005).

Patients with CAR often describe progressive, painless visual loss, over weeks to months, with photosensitivity, peripheral and ring scotomata, and flickering, light-induced glare. Clinically, evidence of cone- and rod-mediated abnormalities are seen on electroretinogram, and while initially normal, fundoscopic exam may demonstrate arteriolar narrowing (Damek 2005). Several antiretinal antibodies are associated with CAR, the most common and well-characterized being anti-recoverin, usually associated with underlying SCLC (Bataller et al. 2004). Recoverin is found in photoreceptor cells, and is thought to modulate dark and light adaptation through a calcium-dependent mechanism. Pathogenesis appears to relate directly to the anti-recoverin antibody, as it induces retinal cell apoptotic death in vitro (Shiraga et al. 2002), and may be found in the aqueous humor of CAR patients (Ohguro et al. 2002). Anti-enolase, a second commonly encountered antibody, is, unlike anti-recoverin, found in non-paraneoplastic retinopathy as well (Adamus et al. 2004). Other target antigens include tubby-like protein, photoreceptor cell-specific nuclear receptor, and polypyrimidine-tract-binding protein-like protein. Treatment, comprising tumor resection and immune suppression, including high-dose steroids and alemtuzumab (Campath), a monoclonal protein directed against CD52, has shown to improve vision in CAR (Alabduljalil et al. 2007).

Symptom onset in MAR, unlike CAR, is usually abrupt and includes night blindness and flickering light phenomena, with normal visual acuity. MAR is almost exclusively associated with melanoma, except for a few reports of a MAR-like syndrome with colon cancer (Jacobson et al. 2001). MAR generally presents months to years after the diagnosis of cancer, usually in the setting of tumor progression. It is believed that MAR antibodies react with retinal bipolar cells, although the exact target antigen is unclear. Recently, mitofilin, a mitochondrial protein, and titin, a striated muscle protein, have been suggested as possible targets. Both are present in retina and tumor cells, and antibodies are seen only in patients with melanoma and MAR (Pföhler et al. 2006). Response to immunosuppressive treatment is usually poor.

Paraneoplastic optic neuropathy rarely occurs alone; it is usually associated with other neurological manifestations including cerebellar degeneration, sensory neuropathy, LEMS, or an encephalomyelopathy. Patients usually present with unilateral, painless, visual loss, progressing to involve both eyes, with spots and flashes before the eyes (Damek et al. 2005). Paraneoplastic optic neuropathy usually occurs in the presence of antibodies to collapsin response mediator protein-5 (CRMP5) in association with SCLC (Cross et al. 2003).

Stiff Person Syndrome

Stiff person syndrome (SPS) is characterized by the gradual onset of stiffness and rigidity, initially in axial muscles, and progressing to proximal limb muscles, primarily involving the legs. Continuously contracting antagonist muscles, described as rock-hard or board-like to palpation, produce a rigid posture, making ambulation difficult, resulting in frequent falls. This muscle rigidity is typically absent during sleep. Patients experience sudden, painful muscle spasms, usually precipitated by touch or involuntary movement, sudden loud noise, or emotional stress. Because of its unusual presentation, SPS was believed to be a functional disorder and many patients were labeled hysterical.

Diagnosis is based on the clinical picture and electromyography (EMG) findings of continuous motor unit activity in affected muscles, which is, however, indistinguishable from normal voluntary muscle contraction. MRI and CSF

examination is usually normal. Other clues to diagnosis include muscle contraction in response to electrical stimulation, termed spasmodic reflex myoclonus, and a dramatic response to diazepam in relieving the stiffness. Differential diagnosis includes tetanus, hyperekplexia, and myelopathy.

SPS has both a non-paraneoplastic and paraneoplastic variant, both autoimmune. Glutamic acid decarboxylase (GAD) antibodies, found in 80 % of SPS patients, are associated with the non-paraneoplastic variant, usually in patients with other autoimmune diseases, particularly diabetes mellitus type 1 (Solimena et al. 1988). GAD catalyzes the decarboxylation of L-glutamate to γ-aminobutyric acid (GABA), the most common inhibitory neurotransmitter in the brain and spinal cord. Paraneoplastic SPS, seen in 5 % of SPS patients (Alexopoulos et al. 2010), is clinically similar to the non-paraneoplastic form, though arm involvement may be more prominent. Amphiphysin antibodies are most commonly detected in paraneoplastic SPS, usually in breast cancer patients, although SCLC and Hodgkin's disease are also reported (Pittock et al. 2004). Case reports of other antibodies associated with SPS include anti-Ri in a patient with lung cancer (McCabe et al. 2004), anti-gephyrin in a patient with mediastinal cancer (Butler et al. 2000), and rare reports of anti-GAD acting as a paraneoplastic antibody (Schiff et al. 2006).

Symptom response to immunotherapy, and the recent discovery that passive transfer of anti-amphiphysin IgG from patients with SPS produced dose-dependent stiffness in rats, suggests a B-cell-mediated process and direct pathogenesis of the antibody. IVIg was effective in 2 placebo-controlled studies in treating the non-paraneoplastic form of SPS associated with anti-GAD antibodies (Dalakas et al. 2001). The evidence for IVIg in paraneoplastic SPS is less substantial, only demonstrated in a few case reports. However, given that both GAD and amphiphysin are part of the presynaptic GABA/glycine inhibitory synapse and likely have similar disease-causing mechanisms, it is reasonable to suspect IVIg, in combination with rigorous treatment of the underlying cancer, may be effective in paraneoplastic SPS as well and may reduce or eliminate the need for benzodiazepines, such as diazepam and clonazepam.

Cross-References

- ▶ Cancer and Dermatomyositis
- ▶ Cancer and Neuropathies
- ▶ Paraneoplastic Neurological Syndromes, Overview

References

Adamus G, Ren G, Weleber RG. Autoantibodies against retinal proteins in paraneoplastic and autoimmune retinopathy. BMC Ophthalmol. 2004;4:5–13.

Alabduljalil T, Behbehani R. Paraneoplastic syndromes in neuro-ophthalmology. Curr Opin Opthalmol. 2007;18:463–9.

Alexopoulos H, Dalakas MC. A critical update on the immunopathogenesis of stiff person syndrome. Eur J Clin Invest. 2010;40(11):1018–25.

Bataller L, Dalmau J. Neuro-ophthalmology and paraneoplastic syndromes. Curr Opin Neurol. 2004;17:3–8.

Bataller L, Graus F, Saiz A, et al. Clinical outcome in adult onset idiopathic or paraneoplastic opsoclonus-myoclonus. Brain. 2001;124:437–43.

Bataller L, Galiano R, Garcia-Escrig M, et al. Reversible paraneoplastic limbic encephalitis associated with antibodies to the AMPA receptor. Neurology. 2010;74:265–7.

Blaes F, Fühlhuber V, Korfei M, et al. Surface-binding autoantibodies to cerebellar neurons in opsoclonus syndrome. Ann Neurol. 2005;58:313–7.

Boronat A, Sabater L, Saiz A, et al. $GABA_B$ receptor antibodies in limbic encephalitis and anti-GAD-associated neurologic disorders. Neurology. 2011;76:795–800.

Butler MH, Hayashi A, Ohkoshi N, et al. Autoimmunity to gephyrin in stiff-man syndrome. Neuron. 2000;26:307–12.

Cross SA, Salomao DR, Parisi JE, et al. Paraneoplastic optic neuritis with retinitis defined by CRMP-5-IgG. Ann Neurol. 2003;54:38–50.

Dalakas MC, Fujii M, Li M, et al. High-dose intravenous immunoglobulin for stiff-person syndrome. N Engl J Med. 2001;345:1870–6.

Dalmau J, Rosenfeld MR. Paraneoplastic syndromes of the CNS. Lancet Neurol. 2008;7:327–40.

Dalmau J, Furneaux HM, Gralla RJ, et al. Detection of the anti-Hu antibody in the serum of patients with small cell lung cancer: a quantitative western blot analysis. Ann Neurol. 1990;27:544–52.

Dalmau J, Graus F, Villarejo A, et al. Clinical analysis of anti-Ma2 associated encephalitis. Brain. 2004;127: 1831–44.

Dalmau J, Lancaster E, Martinez-Hernandez E, et al. Clincal experience and laboratory investigation in patients with anti-NMDAR encephalitis. Lancet Neurol. 2011;10:63–74.

Damek DM. Paraneoplastic retinopathy/optic neuropathy. Curr Treat Options Neurol. 2005;7:57–67.

Darnell RB. Onconeural antigens and paraneoplastic neurologic disorders: at the intersection of cancer, immunity, and the brain. Proc Nat Acd Sci USA. 1996;93:4529–36.

Darnell RB. Paraneoplastic neurologic disorders. Arch Neurol. 2004;61:30–2.

Darnell RB, Posner JB. Paraneoplastic syndromes affecting the nervous system. Semin Oncol. 2006;33: 270–98.

Gorman MP. Update on diagnosis, treatment, and prognosis in opsoclonus-myoclonus-ataxia syndrome. Curr Opin Pediatr. 2010;22:745–50.

Graus F, Saiz A, Lai M, et al. Neuronal surface antigen antibodies in limbic encephalitis: clinical-immunologic associations. Neurology. 2008;71(12): 930–6.

Graus F, Saiz A, Dalmau J. Antibodies and neuronal autoimmune disorders of the CNS. J Neurol. 2010;257:509–17.

Gultekin SH, Rosenfeld MR, Voltz R, et al. Paraneoplastic limbic encephalitis: neurological symptoms, immunological findings and tumor association in 50 patients. Brain. 2000;123:1481–94.

Hammack J, Kotanides H, Rosenblum MK, et al. Paraneoplastic cerebellar degeneration II. Clinical and immunologic findings in 21 patients with Hodgkin's disease. Neurology. 1992;42:1938–43.

Irani SR, Vincent A. NMDA receptor antibody encephalitis. Curr Neurol Neurosci Rep. 2011. [Epub ahead of print]

Jacobson DM, Adamus G. Retinal anti-bipolar cell antibodies in a patient with paraneoplastic retinopathy and colon cancer. Am J Ophthalmol. 2001;131:806–8.

Lawn ND, Westmoreland BF, Kiely MJ, et al. Clinical, magnetic resonance imaging, and electroencephalographic findings in paraneoplastic limbic encephalitis. Mayo Clin Proc. 2003;78:1363–8.

Mason WP, Graus F, Lang B, et al. Small-cell lung cancer, paraneoplastic cerebellar degeneration and the Lambert-Eaton myasthenic syndrome. Brain. 1997;120:1279–300.

McCabe DJH, Turner NC, Chao D, et al. Paraneoplastic "stiff person syndrome" with metastatic adenocarcinoma and anti-Ri antibodies. Neurology. 2004;62:1402–4.

Musunuru K, Darnell RB. Paraneoplastic neurologic disease antigens. Annu Rev Neurosci. 2001;24:239–62.

Ohguro H, Maruyama I, Nakazawa M, et al. Antirecoverin antibody in the aqueous humor of patients with cancer-associated retinopathy. Am J Ophthalmol. 2002;134: 605–7.

Pföhler C, Preuss KD, Tilgen W, et al. Mitofilin and titin as target antigens in melanoma-associated retinopathy. Int J Cancer. 2006;120:788–95.

Pittock SJ, Lucchinetti CF, Lennon VA. Anti-neuronal nuclear autoantibody type 2: paraneoplastic accompaniments. Ann Neurol. 2003;53:580–7.

Pittock SJ, Kryzer TJ, Lennon VA. Paraneoplastic antibodies coexist and predict cancer, not neurological syndrome. Ann Neurol. 2004;56:715–9.

Pranzatelli MR, Tate ED, Travelstead AL, et al. Rituximab (anti-CD20) adjunctive therapy for opsoclonus-myoclonus syndrome. J Pediatr Hematol Oncol. 2006;28:585–93.

Rosenfeld MR, Dalmau J. Update on paraneoplastic and autoimmune disorders of the central nervous system. Semin Neurol. 2010;30:320–31.

Rudnick E, Khakoo Y, Antunes NL, et al. Opsoclonus-myoclonus-ataxia syndrome in neuroblastoma: clinical outcome and antineural antibodies – a report from the children's cancer group study. Med Pediatr Oncol. 2001;36:612–22.

Schiff D, Dalmau J, Myers DJ. Anti-GAD antibody positive stiff-limb syndrome in multiple myeloma. J Neurooncol. 2003;65:173–5.

Shiraga S, Adamus G. Mechanism of CAR syndrome: anti-recoverin antibodies are the inducers of retinal call apoptotic death via the caspase 9- and caspase 3-dependent pathway. J Neuroimmunol. 2002;132: 72–82.

Sillevis Smitt P, Kinoshita A, De Leeuw B, et al. Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. N Eng J Med. 2000;342:21–7.

Solimena M, Folli F, Denis-Donini S, et al. Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. N Engl J Med. 1988;318:1012–20.

Tanaka K, Ding X, Tanaka M. Effects of antineuronal antibodies from patients with paraneoplastic neurological syndrome on primary cultured neurons. J Neurol Sci. 2004;217:25–30.

Tüzün E, Dalmau J. Limbic encephalitis and variants: classification, diagnosis, and treatment. Neurologist. 2007;13:261–71.

Verschuuren J, Chuang L, Rosenblum MK, et al. Inflammatory infiltrates and complete absence of Purkinje cells in anti-Yo-associated paraneoplastic cerebellar degeneration. Acta Neuropathol. 1996;91:519–25.

Vincent A, Buckley C, Schott JM, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. Brain. 2004;127:701–12.

Widdess-Walsh P, Tavee JO, Schuele S, et al. Response to intravenous immunoglobulin in anti-Yo associated paraneoplastic cerebellar degeneration: case report and review of the literature. J Neurooncol. 2003;63:187–90.
Wong A. An update on opsoclonus. Curr Opin Neurol. 2007;20:24–31.

Cancer and Thrombosis

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Synonyms

Deep vein thrombosis; DVT; LMWH; Low molecular weight heparin; PE; Pulmonary embolism; SC; Subcutaneous; TF; Tissue factor; UFH; Unfractionated heparin; Vascular endothelial growth factor; VEGF; Venous thromboembolism; VTE

Definition

Thrombosis is defined as presence of a blood clot in a vein or an artery. If a part of the obstructing clot dissociates and travels in the blood stream, it is termed as "thromboembolism." Venous thromboembolism is one of the common causes of cancer-related morbidity.

Introduction

The relationship between cancer and venous thromboembolism (VTE) has been recognized for almost two centuries. Historically, the French physicians, Bouillaud and Trousseau, are credited with initially describing the relationship between VTE and cancer. Multiple studies have provided considerable evidence for a two-way clinical association between VTE and cancer. While the risk for arterial thrombosis may also be increased in the setting of cancer, this entry will focus on the relationship of malignancy and VTE.

VTE is a common cause of morbidity and mortality in patients with cancer. Patients with cancer are at 2–20-fold higher risk of VTE than patients without cancer, and on average, 15 % of patients develop deep vein thrombosis (DVT) or pulmonary embolism (PE) during the clinical course of their cancer (Chew et al. 2006). The MEGA (Multiple Environmental and Genetic Assessment) study clearly indicated that patients with cancer have an elevated risk for VTE, particularly during the first few months after diagnosis and in the presence of distant metastases (Blom et al. 2005). Conversely, the risk for the diagnosis of cancer seems elevated for at least 2 years after a first episode of idiopathic VTE (Murchison et al. 2004). The data from several large prospective observational and retrospective studies suggest that mortality rate was significantly and consistently greater among cancer patients who developed VTE as compared to patients who did not (Khorana et al. 2007).

Pathophysiology and Risk Factors for Thrombosis in Cancer

Hemostatic and Fibrinolytic Activation

The hemostatic system has been shown to be highly activated in most cancers. Even in the absence of clinical thrombosis, the majority of patients with cancer have increased levels of coagulation factors V, VIII, IX, and XI, as well as increased levels of markers of hemostatic activation (e.g., thrombin-antithrombin; prothrombin fragment 1,2; fibrinopeptide; and D-dimer) (Hoffman et al. 2001). In addition, patients with disseminated malignancies seem to have deficient activity of von Willebrand factor

(vWF)-cleaving protease (ADAMTS13), resulting in unusually large vWF multimers, a key adhesive protein involved in primary hemostasis (Oleksowicz et al. 1999). Many tumors have shown an abnormal expression of high levels of the procoagulant molecule tissue factor (TF) (Rickles and Brenner 2008) and may express an additional cysteine protease cancer procoagulant (Falanga and Gordon 1985). In addition to the expression of TF, tumor cells enhance coagulation by expressing proteins that regulate the fibrinolytic system, including plasminogen activators and plasminogen-activator receptor, leading to an imbalance of fibrinolysis. Acute promyelocytic leukemia is notorious for causing both coagulopathy and hypercoagulable states, with leukemic cells with TF on their cell surface driving the coagulation disarray.

Stasis

At the organism level, malignancy, its complications, and treatments often lead to debility in patients associated with increased rates of sedentary behavior and decreased mobility (Osborne et al. 2008). These directly lead to stasis within the veins of the lower extremities, the sites of most thrombus formation. Venous catheters also lead to local disturbance in blood flow, which is associated with increased rates of thrombosis at the sites of catheters commonly associated with malignancy (Aw et al. 2012). Solid tumors, and lymphomas with large mass effect, may compress vessels and lead directly to vascular stasis, increasing the risk of clotting distal to the obstruction. Most commonly this is seen in the SVC-syndrome associated with mediastinal lymphomas, germ cell tumors, and primary cancers of the thoracic organs. Abdominal and lower extremity veins may be affected by any large, intra-abdominal tumor. Renal cell tumors present a unique situation, as vascular invasion by the tumor itself occurs, occasionally with extension as far as through the right atrium and into the pulmonary vasculature. Differentiating thrombosis from tumor may be difficult in these situations. Hepatobiliary tumors are often associated with portal vein thrombosis, both in the setting of cirrhosis and from portal vein flow reversal.

Vascular Damage

The vasculature is also intimately linked to both the oncologic process and its associated hypercoagulable state. As tumors grow, new vessel growth is required to furnish oxygen and other nutrients, as well as to clear the metabolic waste associated with highly active tumor cells. Cancer therefore stimulates new vessel growth via release of factors such as VEGF, which recruit endothelial cells for new vessel growth (albeit relatively disorganized and ineffectual, leading to the hypoxic and acidic microenvironment found in most tumors beyond a certain size). The same inflammatory mediators that lead to activation of the hemostatic system also lead to widespread activation of the vascular endothelium, which itself becomes a nidus for propagation of the coagulation cascade and ultimately thrombogenesis.

Central catheters and chemotherapeutic agents (see below) are also associated with vascular damage (Bona 1999). The direct physical irritation of blood vessels by central catheters leads to endothelial damage and clot formation and may lead to morbidity and mortality (Aw et al. 2012). Indwelling catheters are associated with a 27–67 % incidence of catheter-associated DVT (Bona 1999; Verso and Agnelli 2003). Chemotherapeutic agents are distributed to the tissues of the body by the vasculature, and these toxic agents may lead to endothelial activation. Some chemotherapeutic agents also appear to directly activate other components of the blood vessels (Otten et al. 2004), as can be seen with cisplatin treatment and arterial events (Moore et al. 2011).

Inflammation and Host Response

The close interactions between the inflammatory/immune system and the hemostatic system are a large part of what underlies the hypercoagulability found and associated with malignancy. Inflammatory mediators are produced by the immune effector cells in response to malignancy as part of the mechanisms to control and eradicate the rogue cancer cells. The hemostatic and inflammatory systems are linked most closely at the start of the intrinsic coagulation cascade, where factor XII is converted to its active form

by multiple members of the inflammatory system, including kininogen. In this way, besides TF acting on the extrinsic pathway, the intrinsic pathway is also recruited to amplify the coagulation cascade and thereby exacerbate the prothrombotic state (Rickles and Brenner 2008). Tumor-infiltrating macrophages may lead to downstream production of TF, and cytokine response to tumor and/or therapy may increase thrombotic tendencies.

Inflammation is also associated with both chemotherapeutic treatment and the infections caused by the immune-compromised state associated with chemotherapeutic treatments. Indeed, some of the highest rates of thrombosis in cancer patients are found during periods of hospitalization when patients are both immobilized, and the immune system is mounting high levels of inflammatory responses (Esmon 2003).

Cancer therapy also increases the risk for VTE, including surgery, chemotherapy, anti-angiogenic therapy, and hormonal therapy. Oncologic surgery is associated with an increased rate of VTE compared to noncancer surgery (White et al. 2003; Clagett and Reisch 1988). Chemotherapy has been known to increase the risk of thrombosis for some time (Lee and Levine 1999; Levine et al. 1988). In a prospective, multicenter, observational study, the overall incidence of VTE in an ambulatory population starting new chemotherapy was 1.93 % with a median follow-up of 2.4 months. The rate of VTE observed in this study (0.8 % per month) was substantially in excess of the estimated rate of approximately 0.04 % per month for the entire cancer population (Blom et al. 2004). Similarly, anti-angiogenic therapies may have significant effects on VTE incidence (Nalluri et al. 2008). Hormonal therapy is also recognized to increase the risk for VTE (Lee and Levine 1999; Ehdaie et al. 2011).

Specific factors increasing the risk for VTE include cancer type (e.g., pancreatic cancer, brain cancer, lymphoma) and stage (Levitan et al. 1999; Blom et al. 2005). In addition to the histology and type of cancer, there are various other factors that contribute towards the development of VTE in the setting of malignancy. Such factors have been identified using data from population-based databases, registries, hospital records, retrospective cohorts, prospective observational studies, and clinical trials (Table 1).

Cancer and Thrombosis, Table 1 Risk factors and biomarkers

Risk factors and biomarkers for development of VTE in cancer patients	Selected references
Extent of disease and metastasis	Levitan et al. (1999), Blom et al. (2005)
Chemotherapy	Blom et al. (2004), Lee and Levine (1999), Levine et al. (1988)
Hormonal therapy	Lee and Levine (1999), Ehdaie et al. (2011)
Anti-angiogenic therapy	Nalluri et al. (2008)
Central venous catheters	Aw et al. (2012), Verso and Agnelli (2003)
Thrombocytosis	Khorana et al. (2008)
Leukocytosis	Khorana et al. (2008)
Anemia	Khorana et al. (2008)
High levels of tissue factor (TF) expression or elevated levels of circulating TF	Belting et al. (2005)
Elevated soluble P-selectin level	Ay et al. (2008)
Histology of cancer	Levitan et al. (1999)
D-dimer	Ay et al. (2009)
Thrombin-antithrombin complex	Kakkar et al. (1995)
Microparticles (derived from platelets, megakaryocytes, or leukocytes)	Campello et al. (2011)
Erythropoiesis-stimulating agents	Bennett et al. (2008)
Surgery	White et al. (2003), Clagett and Reisch (1988)

Although the development of VTE in a patient with known cancer is the most common presentation, in some patients VTE may precede the diagnosis of malignancy (Murchison et al. 2004). Both retrospective and prospective studies have identified this phenomenon (Nordstrom et al. 1994; Sorensen et al. 1998). The variation in clinical presentation is likely due to the heterogeneous biology of different tumor types and reflects the limitations of detection or available diagnostic methods. Accumulating evidence now

suggests that oncogenic events also trigger activation of the coagulation cascade, leading to a thrombotic environment that not only manifests as VTE but also promotes the growth of the malignancy (Rickles and Brenner 2008).

Clinical Risk Stratification

It is increasingly recognized that ambulatory patients (especially those receiving systemic therapy) are at increased risk for VTE, and the vast majority of patients with cancer are in the ambulatory setting. The five-item Khorana model has been validated, using data from the Vienna Cancer and Thrombosis Study in a much broader range of patients (Khorana et al. 2008). The major advantage of this model is the easy availability of these common clinical markers, while the major limitation is the generalizability of the results. This model identifies about 7 % of cancer patients receiving chemotherapy in the ambulatory setting as high risk of VTE. Furthermore, the model is able to identify about 30 % of patients as low risk for VTE. This model is also being tested in an ongoing study, funded by the National Heart, Lung, and Blood Institute (www.clinicaltrials.gov NCT00876915).

Treatment and Secondary Prophylaxis of VTE in Cancer

Venous thromboembolic disease is a leading cause of death among cancer patients (Khorana et al. 2007). In addition, patients with cancer and thrombosis are at increased risk of VTE recurrence compared with noncancer patients and at increased risk of death due to malignancy compared to cancer patients without VTE (Levitan et al. 1999; Sorensen et al. 2000).

The recommended treatment for cancer-associated thrombosis is low molecular weight heparin (LMWH). For the initial treatment phase, post hoc data from randomized trials suggest comparable efficacy between unfractionated heparin (UFH) and LMWH. Based upon several prospective studies (see Table 2), recommendations for the treatment of proximal lower extremity DVT and/or pulmonary embolism in the setting of active cancer are with the use of extended LMWH therapy (Lyman et al. 2007; Geerts et al. 2008), and this therapy appears to be feasible. Some of the new anticoagulants including dabigatran and rivaroxaban are comparable to warfarin in efficacy and safety in a general patient population, but these trials include only a minority of cancer patients and have not been compared directly with LMWH.

Primary Prophylaxis

Primary anticoagulant prophylaxis is recommended in all oncology patients admitted to the hospital for surgical or medical reasons (Lyman et al. 2007). Although there are data for UFH, low molecular weight heparin (LMWH), fondaparinux, and warfarin for primary prophylaxis, contemporary studies have largely studied LMWH (Geerts et al. 2008). The ACCP, ASCO, NCCN, and ESMO guidelines recommend antithrombotic prophylaxis for cancer surgery for at least 7–10 days postoperatively (Lyman et al. 2007; Geerts et al. 2008). The benefit of extended prophylaxis for VTE following cancer surgery was first demonstrated by the ENOXACAN II study (ENOXACAN study group 1997). The role of antithrombotic prophylaxis in the prevention of central venous catheter-related thrombosis is controversial, and the international guidelines do not recommend prophylaxis for this indication (Lyman et al. 2007; Geerts et al. 2008).

The benefit of antithrombotic prophylaxis in ambulatory cancer patients receiving chemotherapy has been evaluated in several studies that have demonstrated efficacy. An early high-quality study utilized low-dose warfarin to decrease VTE in women receiving therapy for breast cancer (Levine et al. 1994). More recently, prophylactic treatment with LMWH in

Cancer and Thrombosis, Table 2 Clinical trials for long-term use of LMWH in patients with VTE and cancer

Study	Trial design	Arms	Results	Comments
CANTHANOX (Meyer et al. 2002)	Randomized open label multi-institutional study	1. Enoxaparin	For enoxaparin group: 8 (11.3 %) had PE, 19 (26.8 %) had DVT, and 44 (62 %) had both DVT/PE	No statistically significant difference in combined primary outcome between enoxaparin group (7/67, 10.5 %) and warfarin group (15/71, 21.1 %, p = 0.09). Use of long-term LMWH in cancer patients was found to be more effective and safe
		2. Warfarin	For warfarin group: 11 (14.7 %) had PE, 25 (33.3 %) had DVT, and 39 (52 %) had both	
ONCENOX (Deitcher et al. 2006)	Randomized open label multi-institutional trial	1. Enoxaparin 1 mg/kg SC 2 times/day for 5 days, followed by 1 mg/kg SC 1 time/day for 175 days	Regarding primary outcomes, compliance rates were 97.9 % and 97 % for the two enoxaparin groups, and 90.1 % for warfarin group	Treatment with enoxaparin was feasible, generally well tolerated, and effective for a 180-day period in secondary prevention of VTE in cancer patients
		2. Enoxaparin 1 mg/kg SC 2 times/day for 5 days, followed by 1.5 mg/kg SC 1 time/day for 175 days	Secondary results for efficacy showed no difference in recurrent VTE rates for enoxaparin 1 mg/kg SC once daily group (1/29, 3.4 %), enoxaparin 1.5 mg/kg SC once daily group (1/32, 3.1 %), or warfarin group (2/30, 6.7 %, no p-value)	
		3. Enoxaparin 1 mg/kg SC twice daily for at least 5 days, until they reached therapeutic INR. Warfarin was started on day two of treatment and continued until patients received 180 total days of anticoagulation		
CLOT (Lee et al. 2003)	Randomized multi-institutional study	1. Dalteparin + coumarin derivative	27/336 (8.0 %) patients in dalteparin group had recurrent VTE, compared to 53/336 (15.8 %) patients in oral anticoagulant group (hazard ratio, 0.48; p = 0.002)	Dalteparin was more effective than oral anticoagulant in reducing the risk of recurrent VTE, without increasing bleeding risk
		2. Dalteparin alone		
LITE (Hull et al. 2007)	Randomized open label multi-institutional study	1. Tinzaprin	Of 737 patients, 18/369 (4.8 %) receiving tinzaparin had recurrent VTE at 3 months compared with 21/368 (5.7 %) receiving Vitamin K antagonist	Tinzaparin is similar in effectiveness to the usual-care vitamin K antagonist treatment for preventing VTE in a broad population of patients
		2. Vitamin K antagonist		
Tinzaparin prospective study (Tagawa et al., 2010)	Single-arm dual-center prospective study including biomarkers	Tinzaparin for 6 (up to 12) months at investigator discretion	11.3 % recurrent VTE rate (including unsuspected VTE on staging imaging); 3 % major bleeding	Increase in D-dimer at 1 month was associated with recurrent VTE (p = 0.009)

Cancer and Thrombosis, Table 3 Clinical trials of prophylactic anticoagulation in cancer patients receiving chemotherapy

Trial name	Patient population	Treatment arms	No. of patients	Results	Comments
Levine et al. (1994)	Women with breast cancer receiving chemotherapy	1. Warfarin 2. Placebo	311	There were 6 DVT, 1 PE in placebo group and 1 PE in warfarin group, a relative risk reduction of about 85 % ($p = 0.031$). Bleeding occurred in 2 placebo recipients and 1 warfarin-treated patient	Very-low-dose warfarin is a safe and effective method for prevention of VTE in patients
PROTECHT (Agnelli et al. 2009)	Patients with lung, breast, gastrointestinal, ovarian, or head and neck cancer receiving chemotherapy	1. Nadroparin 2. Placebo	1,150	Incidence of symptomatic venous and arterial thromboembolic events in nadroparin arm was 2 % versus 3.9 % in placebo group	High rates of VTE in lung (8.8 %) and pancreatic cancer patients (5.9 %) suggest need for more trials in these groups
CONKO 004 (Riess et al. 2009)	Advanced pancreatic cancer patients receiving chemotherapy	1. Enoxaparin 2. Observation	312	Sixty-five percent relative risk reduction of symptomatic VTE following use of enoxaparin 5 % versus 14.5 % for observation group	No overall survival difference between two groups
FRAGEM (Maraveyas et al. 2012)	Advanced pancreatic cancer patients eligible to receive gemcitabine	1. Gemcitabine + Dalteparin 2. Gemcitabine alone	123	Incidence of all-type VTE during the entire follow-up was 12 % in dalteparin group versus 28 % in those receiving chemotherapy alone	No significant difference in survival or bleeding events
SAVE-ONCO (Agnelli et al. 2012)	Metastatic or locally advanced solid tumors who were beginning to receive a course of chemotherapy	1. Semuloparin 2. Placebo		Occurrence of VTE was 1.2 % in semuloparin group versus 3.4 % in placebo group; HR 0.36; 95 % CI 0.21–0.60	No significant survival benefit found at 1-year follow-up

ambulatory patients receiving chemotherapy has been demonstrated to be efficacious with risk reductions VTE (see Table 3). However, it remains to be seen if these studies will change clinical practice, and no prospective studies have examined cost-effectiveness. Currently, VTE prophylaxis in ambulatory patients is recommended only for patients with multiple myeloma receiving thalidomide or lenalidomide-based combination chemotherapy (Lyman et al. 2007).

While not the subject of this entry, anticoagulants may have anticancer properties. Several retrospective studies pointing towards a survival benefit independent of VTE incidence led to a number of prospective studies with response or survival endpoints. Overall, pooled data point towards a reduction in the risk of death in subjects receiving anticoagulation therapy (Akl et al. 2007, 2010).

Conclusion

Venous thromboembolism is a significant source of morbidity and mortality in patients with cancer. Tumor factors, host factors, and treatment lead to the increased risk of VTE in the setting of

malignancy. Patients with cancer and thrombosis should be viewed as a special population and treated differently than similar patients with thrombosis in the absence of malignancy. The onset of idiopathic VTE may be the first manifestation of malignancy. In high-risk settings particularly, such as hospitalized and perioperative situations, VTE prophylaxis is indicated. Primary VTE prophylaxis in the ambulatory setting decreases the incidence of thrombosis, though the cost-effectiveness of this approach has not yet been demonstrated. Lastly, in addition to decreasing the risk of onset or recurrence of VTE, anticoagulants (in particular LMWH) may have antitumor properties which may lead to prolonged survival in the setting of malignancy.

References

Agnelli G, Gussoni G, Bianchini C, et al. Nadroparin for the prevention of thromboembolic events in ambulatory patients with metastatic or locally advanced solid cancer receiving chemotherapy: a randomised, placebo-controlled, double-blind study. Lancet Oncol. 2009;10:943.

Agnelli G, George DJ, Kakkar AK, et al. Semuloparin for thromboprophylaxis in patients receiving chemotherapy for cancer. N Engl J Med. 2012;366:601.

Akl EA, van Doormaal FF, Barba M, et al. Parenteral anticoagulation for prolonging survival in patients with cancer who have no other indication for anticoagulation. Cochrane Database Syst Rev. 2007a;3:CD006652.

Akl EA, Kamath G, Kim SY, et al. Oral anticoagulation for prolonging survival in patients with cancer. Cochrane Database Syst Rev. 2010;8(12):CD006466. doi:10.1002/14651858.CD006466.pub2.

Aw A, Carrier M, Koczerginski J, McDiarmid S, Tay J. Incidence and predictive factors of symptomatic thrombosis related to peripherally inserted central catheters in chemotherapy patients. Thromb Res. 2012;130(3):323–6.

Ay C, Simanek R, Vormittag R, et al. High plasma levels of soluble P-selectin are predictive of venous thromboembolism in cancer patients: results from the Vienna Cancer and Thrombosis Study (CATS). Blood. 2008;112:2703–8.

Ay C, Vormittag R, Dunkler D, et al. D-dimer and prothrombin fragment 1 + 2 predict venous thromboembolism in patients with cancer: results from the Vienna Cancer and Thrombosis Study. J Clin Oncol. 2009;27:4124–9.

Belting M, Ahamed J, Ruf W. Signaling of the tissue factor coagulation pathway in angiogenesis and cancer. Arterioscler Thromb Vasc Biol. 2005;25:1545–50.

Bennett CL, Silver SM, Djulbegovic B, et al. Venous thromboembolism and mortality associated with recombinant erythropoietin and darbepoetin administration for the treatment of cancer-associated anemia. JAMA. 2008;299:914.

Blom JW, Osanto S, Rosendaal FR. The risk of a venous thrombotic event in lung cancer patients: higher risk for adenocarcinoma than squamous cell carcinoma. J Thromb Haemost. 2004;2:1760–5.

Blom JW, Doggen CJ, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. JAMA. 2005;293:715–22.

Bona RD. Thrombotic complications of central venous catheters in cancer patients. Semin Thromb Hemost. 1999;25:147–55.

Campello E, Spiezia L, Radu CM, Bulato C, et al. Endothelial, platelet, and tissue factor-bearing microparticles in cancer patients with and without venous thromboembolism. Thromb Res. 2011;127:473–7.

Chew HK, Wun T, Harvey D, Zhou H, White RH. Incidence of venous thromboembolism and its effect on survival among patients with common cancers. Arch Intern Med. 2006;166:458–64.

Clagett GP, Reisch JS. Prevention of venous thromboembolism in general surgical patients. Results of meta-analysis. Ann Surg. 1988;208:227–40.

Deitcher SR, Kessler CM, Merli G, Rigas JR, Lyons RM, Fareed J. Secondary prevention of venous thromboembolic events in patients with active cancer: enoxaparin alone versus initial enoxaparin followed by warfarin for a 180-dayvperiod. Clin Appl Thromb Hemost. 2006;12:389–96.

Ehdaie B, Atoria CL, Gupta A, et al. Androgen deprivation and thromboembolic events in men with prostate cancer. Cancer. 2011. doi:10.1002/cncr.26623.

ENOXACAN Study Group. Efficacy and safety of enoxaparin versus unfractionated heparin for prevention of deep vein thrombosis in elective cancer surgery: a double-blind randomized multicentre trial with venographic assessment. Br J Surg. 1997;84:1099–103.

Esmon C. Inflammation and thrombosis. J Thromb Haemost. 2003;1:1343–8.

Falanga A, Gordon SG. Isolation and characterization of cancer procoagulant: a cysteine proteinase from malignant tissue. Biochemistry. 1985;24:5558–67.

Geerts WH, Bergqvist D, Pineo GF, et al. Prevention of venous thromboembolism: American College of chest physicians evidence-based clinical practice guidelines (8th edition). Chest. 2008;133(6 Suppl):381S–453.

Hoffman R, Haim N, Brenner B. Cancer and thrombosis revisited. Blood Rev. 2001;15:61–7.

Hull RD, Pineo GF, Brant RF, et al. Self-managed long-term low-molecular-weight heparin therapy: the balance of benefits and harms. Am J Med. 2007;120:72–82.

Kakkar AK, DeRuvo N, Chinswangwatanakul V, Tebbutt S, Williamson RC. Extrinsic-pathway activation in cancer with high factor VIIa and tissue factor. Lancet. 1995;346:1004–5.

Khorana AA, Francis CW, Culakova E, Kuderer NM, Lyman GH. Thromboembolism is a leading cause of death in cancer patients receiving outpatient chemotherapy. J Thromb Haemost. 2007;5:632–4.

Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. Blood. 2008;111:4902–7.

Lee AY, Levine MN. The thrombophilic state induced by therapeutic agents in the cancer patient. Semin Thromb Hemost. 1999;25:137–45.

Lee AY, Levine MN, Baker RI, et al. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. N Engl J Med. 2003;349:146–53.

Levine MN, Gent M, Hirsh J, et al. The thrombogenic effect of anticancer drug therapy in women with stage II breast cancer. N Eng J Med. 1988;318:404–7.

Levine M, Hirsh J, Gent M, et al. Double-blind randomised trial of a very-low-dose warfarin for prevention of thromboembolism in stage IV breast cancer. Lancet. 1994;343:886–9.

Levitan N, Dowlati A, Remick SC, et al. Rates of initial and recurrent thromboembolic disease among patients with malignancy versus those without malignancy. Risk analysis using Medicare claims data. Medicine (Baltimore). 1999;78:285–91.

Lyman GH, Khorana AA, Falanga A, et al. American Society of Clinical Oncology guideline: recommendations for venous thromboembolism prophylaxis and treatment in patients with cancer. J Clin Oncol. 2007;25:5490–505.

Maraveyas A, Waters J, Roy R, et al. Gemcitabine versus gemcitabine plus dalteparin thromboprophylaxis in pancreatic cancer. Eur J Cancer. 2012; 48(9):1283–92.

Meyer G, Marjanovic Z, Valcke J, et al. Comparison of low-molecular-weight heparin and warfarin for the secondary prevention of venous thromboembolism in patients with cancer: a randomized controlled study. Arch Intern Med. 2002;162:1729–35.

Moore RA, Adel N, Riedel E, et al. High incidence of thromboembolic events in patients treated with cisplatin-based chemotherapy: a large retrospective analysis. J Clin Oncol. 2011;29:3466.

Murchison JT, Wylie L, Stockton DL. Excess risk of cancer in patients with primary venous thromboembolism: a national, population-based cohort study. Br J Cancer. 2004;91:92–5.

Nalluri SR, Chu D, Keresztes R, Zhu X, Wu S. Risk of venous thromboembolism with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis. JAMA. 2008;300:2277–85.

Nordstrom M, Lindblad B, Anderson H, Bergqvist D, Kjellstrom T. Deep venous thrombosis and occult malignancy: an epidemiological study. Br Med J. 1994;308:397–894.

Oleksowicz L, Bhagwati N, DeLeon-Fernandez M. Deficient activity of von Willebrand's factor-cleaving protease in patients with disseminated malignancies. Cancer Res. 1999;59:2244–50.

Osborne NH, Wakefield TW, Henke PK. Venous thromboembolism in cancer patients undergoing major surgery. Ann Surg Oncol. 2008;15:3567–78.

Otten HM, Mathijssen J, ten Cate H, et al. Symptomatic venous thromboembolism in cancer patients treated with chemotherapy: an underestimated phenomenon. Arch Intern Med. 2004;164:190.

Rickles FR, Brenner B. Tissue factor and cancer. Semin Thromb Hemost. 2008;34:143–5.

Riess H, Pelzer U, Deutschinoff G, et al. A prospective, randomized trial of chemotherapy with or without the low molecular weight heparin (LMWH) enoxaparin in patients (pts) with advanced pancreatic cancer (APC): results of the CONKO 004 trial (abstract). J Clin Oncol. 2009;27:798s.

Sorensen HT, Mellemkjaer L, Steffensen FH, Olsen JH, Nielsen GL. The risk of a diagnosis of cancer after primary deep venous thrombosis or pulmonary embolism. N Engl J Med. 1998;338:1169–73.

Sorensen HT, Mellemkjaer L, Olsen JH, et al. Prognosis of cancers associated with venous thromboembolism. N Engl J Med. 2000;343:1846–50.

Tagawa ST, Weitz IC, O'Connell CL, et al. Tinzaparin is effective and safe for the treatment and extended secondary prophylaxis in cancer patients with venous thromboembolism. Blood 2010;116:1104.

Verso M, Agnelli G. Venous thromboembolism associated with long-term use of central venous catheters in cancer patients. J Clin Oncol. 2003;21: 3665–75.

White RH, Zhou H, Romano PS. Incidence of symptomatic venous thromboembolism after different elective or urgent surgical procedures. Thromb Haemost. 2003;90:446–55.

CD40

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Synonyms

BP50; CDW40; P50; TNFR superfamily member 5 (*TNFR5*)

Definition

Historical Background

CD40 is a type I integral membrane protein and a member of the tumor necrosis factor receptor (TNFR) family of molecules that functions as a major communication link between antigen-presenting cells (APCs) (B cells, macrophage, and dendritic cells (DCs)) and CD4 T cells. In the years prior to the discovery of CD40, there was widespread appreciation that antigen-specific activation of naïve B cells required contact-dependent interactions with activated T cells. However, only subsequent to demonstrating that contact was independent of cognate MHC interactions did it become clear that B-T cell contact required interactions with membrane-associated factors and not just cytokines (Banchereau et al. 1994; Lederman et al. 1993). CD40 was initially discovered as a B cell surface protein that could drive the proliferation and enhance the differentiation of B cells following stimulation with anti-CD40 antibodies (van Kooten and Banchereau 2000). CD40 expression occurred at all stages of B cell development (except for late-stage plasmablasts), whereas its ligand known as CD154 or CD40L was transiently expressed primarily on activated CD4 T cells. The absolute requirement for CD40-CD40L signaling in humoral immunity was first demonstrated in patients expressing a severe form of immunodeficiency termed X-linked hyper-IgM syndrome (HIGM1). These individuals displayed deficient CD40L expression, which resulted in significantly increased levels of IgM with a corresponding lack of "switched" isotypes (IgG, IgA, or IgE). Also, patients failed to form germinal centers (GCs) in their lymph nodes in response to antigenic challenge. Further work with animal models lacking either functional CD40 or CD40L confirmed that CD40 signals were essential for establishing both humoral and cell-mediated immune responses (Lougaris et al. 2005).

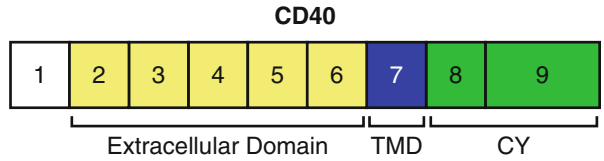
Structure of CD40 and CD40L

The structure of human CD40 was deduced from a cDNA isolated from a B cell expression library and shown to be a type I integral membrane protein comprised of 277 amino acids with a 193-amino-acid extracellular domain and a 62-amino-acid intracellular tail (Fig. 1) (van Kooten and Banchereau 2000). A comparison of the human and mouse forms of the protein uncovered a high level of identity at the amino acid level 62 % and complete conservation of 32 amino acids at the carboxyl terminal end of the protein. Also, all four of the extracellular cysteine residues are highly conserved, suggesting that the folding of the structural regions between mouse and human CD40 are identical. The mouse CD40 gene is located on the distal region of chromosome 2, which is syntenic to human chromosome 20Q11–Q13, harboring the human CD40 gene. CD40 exists as multiple spliced isoforms, and these different forms are correlated with distinct functional responses (Tone et al. 2001).

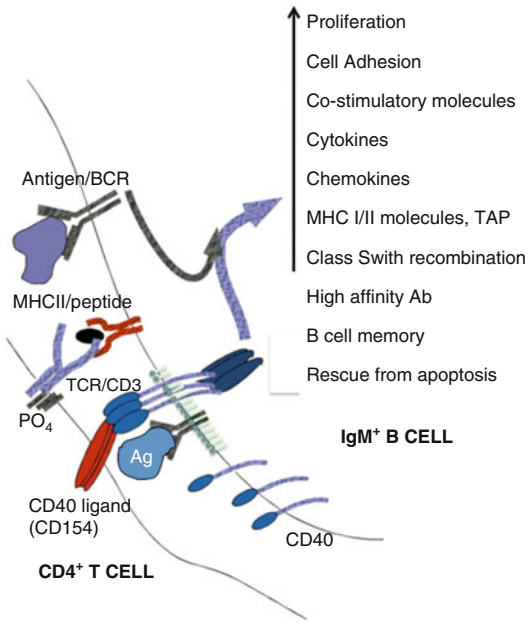
The structure of the CD40L protein was initially identified as a 33–39 kDa type II integral membrane protein that shared a high degree of homology to TNF-α. X-ray crystallographic data structure consisting of a sandwich of two β-sheets with jellyroll topology and revealed a surface topography that was homotrimeric with three-way symmetry. This type of 3-dimensional organization was reminiscent of both TNF-α and LT-α proteins. In addition to the full-length protein, two shorter, soluble versions of the CD40L protein were identified (31 and 18 kDa), and these forms retained their ability to trimerize and deliver biological signals through engaging CD40 (van Kooten and Banchereau 2000).

CD40 and B Cell Function

The initial discovery of CD40 was followed by an intense and productive period of investigation by many laboratories that focused primarily on the characterization of CD40-mediated functional responses in B cells. In vitro experiments revealed that CD40 was required for multiple B cell processes including proliferation, differentiation, and Ig production of both immature and mature B cell subsets (Banchereau et al. 1994; Noelle 1995; van Kooten and Banchereau 2000). In addition, early studies highlighted the critical



CD40, Fig. 1 Molecular organization of human CD40. Exons (1–9) are indicated by *boxes* and depicted as components of the extracellular domain, transmembrane domain (*TMD*), or cytoplasmic domain (*CY*)

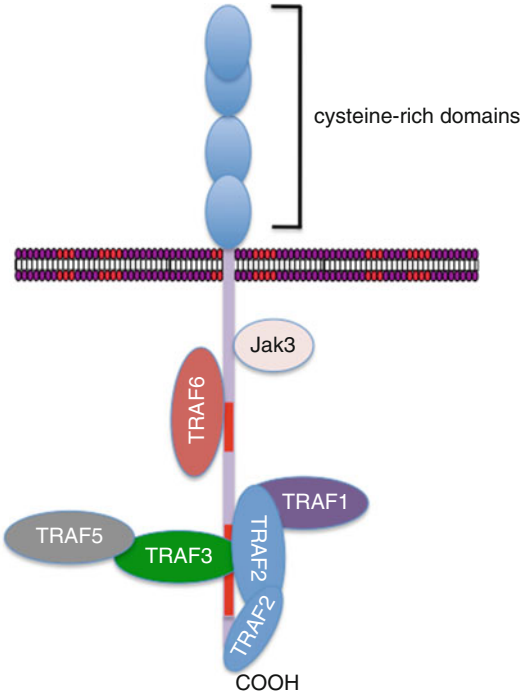


CD40, Fig. 2 CD40 signaling is provided to B cells that have encountered antigen (*Ag*) through the B cell receptor (*BCR*). These signals lead to a multitude of phenotypic changes that form the basis of the humoral immune response

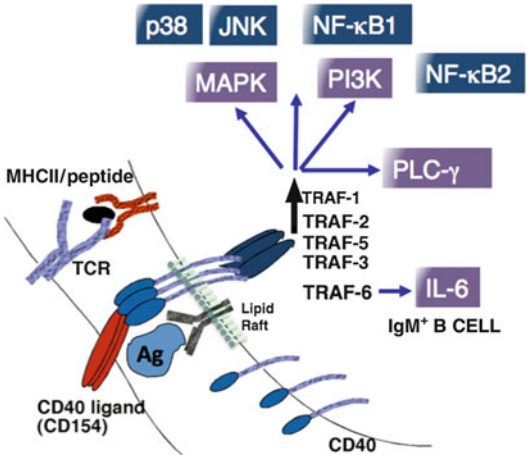
role of CD40 in the B cell maturation program including rescue from apoptosis, GC formation, isotype switching, somatic hypermutation, and B cell selection and development into memory cells. CD40 signals also directly affected cytokine production and expression of adhesion molecules and costimulatory receptors and increased the expression of MHC class I, MHC class II, and TAP transporter molecules (Fig. 2) (van Kooten and Banchereau 2000). However, CD40 signaling was shown to be dispensable for thymus-independent (TI) antibody responses, as evidenced by the fact that responses mounted to TI antigens (TNP-LPS and TNP-Ficoll) were similar between CD40-deficient and wild-type mice (van Kooten and Banchereau 2000). Overall, these findings revealed that CD40 is essential for generating responses to thymus-dependent (TD) antigens, responses that inherently result in the development of B cell memory and the corresponding production of somatically mutated, high-affinity antibodies.

Signaling Through Tumor Necrosis Factor Receptor-Associated Factors (TRAFs)

CD40 is distributed randomly throughout the plasma membrane and upon CD40L engagement clusters into cholesterol- and glycolipid-rich microdomains (termed lipid rafts). These unique membrane structures consist of concentrated pools of signaling molecules and are sites of initiation of signaling pathways for many different receptors including the B cell Receptor (BCR) and the T cell (TCR) receptor (Bishop et al. 2007). CD40 lacks intrinsic signaling capability and therefore is dependent on TNFR-associated factor (TRAF) adapter proteins to transmit CD40-mediated signals. This family of molecules is composed of six members, designated TRAF1 through TRAF6, that function by binding to discrete sites within the conserved carboxyl domain of TNFR family members including CD40 (termed the TRAF domain). Mapping experiments revealed that TRAF1, TRAF2, and TRAF3 bind to overlapping sites within the distal end of the CD40 intracytoplasmic tail that contains the consensus sequence PxQxT. A second, noncanonical binding site for TRAF2 resides adjacent to the carboxyl terminus (Elgueta et al. 2009). In contrast, the TRAF6 consensus site lies in the membrane proximal domain of CD40 (Fig. 3). The TRAF2/3 consensus site is required for the induction of



CD40, Fig. 3 The cytoplasmic domain of CD40 has two major binding sites for TRAF molecules which are situated proximal (TRAF6) and distal (TRAF1, 2, 3, and 5) from the membrane. A second TRAF2 binding site has been identified at the carboxyl end of the protein.



CD40, Fig. 4 Schematic of signaling pathways and transcription factors activated in response to CD40-CD40L engagement

NFκB, mitogen-activated protein kinase (MAPK)-JNK and MAPK-p38 pathways; however, in B cells, TRAF2 is a strong positive regulator and TRAF3 is a negative regulator of the canonical and noncanonical (NF-κB) pathways (Bishop et al. 2007; Elgueta et al. 2009). Accordingly, loss of TRAF3 is associated with an increased incidence of B cell lymphomas in both mice and humans (Bishop and Xie 2007). TRAF1 primarily binds to CD40 indirectly by forming multimers with TRAF2 and TRAF1 can bind directly to CD40 in the absence of TRAF2. TRAF5 does not directly bind to the CD40 tail but forms heterotrimers with TRAF3 upon CD40-CD40L contact. Lastly TRAF6 binding is required for the production of IL-6 by B cells. The binding of TRAF molecules as homo- or heterotrimers or as higher-order oligomers most likely drives the diverse array of downstream effector functions that are the consequence of CD40 signaling.

The engagement of TRAF signaling by CD40 results in the activation of multiple signaling cascades including the MAPK JNK and -p38 phosphoinositide 3-kinase (PI3K), and the phospholipase Cγ (PLCγ) pathways, leading to the induction of multiple signaling pathways including the canonical and noncanonical NF-κB-signaling pathways (Fig. 4). More recent findings suggest that TRAF-independent signaling may occur through the direct binding of Janus family kinase 3 (Jak3) to a consensus site within the intracytoplasmic domain of CD40. Binding of Jak3 induces the phosphorylation of signal transducer and activator of transcription 5 (STAT5). Together, these complex pathways transmit the essential transcription-dependent and transcription-independent signals that underlie CD40-specific functions (Bishop et al. 2007; Elgueta et al. 2009). Numerous studies suggest that the strength and duration of CD40 signals are critical for inducing specific downstream responses. This is observed at the level of different stimuli where anti-CD40 antibodies trigger only a subset of responses compared to trimeric CD40L (Bishop et al. 2007; Elgueta et al. 2009). For example, aggregating CD40 through binding to membrane-bound or trimeric CD40L is required for TRAF6 binding which results in the production of IL-6.

CD40 and the GC Response

It has been recently appreciated that the strength and duration of CD40 signaling have a major impact on the fate of antigen-selected B cells in secondary lymphoid tissues. B cells require CD40 signals to generate GCs as evidenced by the fact that an absence of signal fails to give rise to GC formation. The quality of CD40 signaling markedly influences the differentiation outcome of B cells in the GC such that when a high level of stimulus is provided in the context of an ongoing immune response, B cells are selected to differentiate into plasmablasts and are precluded from entering the GC reaction. However, once B cells are selected to colonize the B cell follicles and initiate GC formation, daughter centrocytes with enhanced BCR affinity for antigen will engage CD40L on follicular T helper cells, resulting in an effective block in Fas-dependent apoptosis through Bcl-XL- and c-FLIP-dependent mechanisms. In contrast, if a B cell expresses a BCR with weak antigen affinity, it fails to receive adequate BCR and CD40 survival signals and is deleted from the repertoire (Benson et al. 2007). The effects of graded levels of CD40 signals have been analyzed for affects in other APCs. Accordingly, strong and weak CD40 signals resulted in phenotypic and functional changes in both macrophage and DCs (Benson et al. 2007).

Expression of CD40 in Other Cell Types

Although early work focused on the critical role of CD40 in B cell biology, it became increasingly clear that CD40 was expressed in a much broader context and by a number of distinct cell types including myeloid cells, DCs, follicular DCs, endothelial cells, fibroblasts, epithelial cells, and keratinocytes (Elgueta et al. 2009; van Kooten and Banchereau 2000). Also, the spectrum of cells expressing CD40L was expanded to include mast cells, basophils, eosinophils, and activated platelets (Elgueta et al. 2009). Thus, the scope of effector functions that were controlled by CD40-CD40L signaling was greatly elaborated to more accurately reflect the expression pattern of these two molecules. Importantly, CD40-CD40L interactions were found to be critical for effective macrophage and DC function and to strongly influence T cell priming and consequently, T cell-mediated functions. The critical role of the receptor-ligand pair in APC function reflects the fact that CD40 signaling directly results in the upregulation of costimulatory molecules CD80 and CD86 on APCs. Ligation of these proteins by CD28 expressed on T cells leads to the induction of effector functions such as CD8 cytotoxic activity and the expression of multiple cytokines and chemokines critical for inflammatory responses (van Kooten and Banchereau 2000). Thus, in addition to roles in humoral immunity, CD40 and CD40L are central players in inflammatory processes and the cell-mediated immune responses to infection (Elgueta et al. 2009; Grewal and Flavell 1997; van Kooten and Banchereau 2000).

CD40 Expression and Macrophage Function

The interpretation of CD40 signals by macrophage and monocytes is known to be highly dependent on the tissue source of cells and most likely reflects the priming effect of the tissue environment (Elgueta et al. 2009). Generally, CD40 ligation of on macrophage/monocytes produce a proinflammatory signature including the production of cytokines (IL-1α and IL-1β, TNF-α, IL-6, and IL-12) and chemokines (MCP-1, RANTES, MIP-1α, and MIP-1β) and the upregulation of MHC class II and costimulatory molecules (CD80 and CD86) (Elgueta et al. 2009). Importantly, CD40 engagement also induces the expression of matrix metalloproteinases and nitric oxide (NO), mediators required for the destruction of damaged or infected cells. Interestingly, treatment of tumor-bearing mice with an agonistic anti-CD40 mAb was found to activate macrophage cytostatic activity, which led to the suppression of tumor cell growth (Loskog and Totterman 2007). Whereas TGF-β, a cytokine that inhibits CD40 responses and is expressed by many different tumor types, is capable of rendering tumor-associated macrophage incapable of mounting an attack on the tumor. Thus, the tissue environment and the nature of the delivery of the CD40 stimulus dictate the cellular outcomes that either can be protective from disease or contribute to disease progression.

The Role of CD40 in Infection

As mentioned earlier, CD40-CD40L interactions are critical for the development of CD8 T cell immunity, which in turn mediates immune responses to intracellular pathogens and tumors. Optimal activation of CD8 T cells requires engagement of the Ag receptor (TCR by Ag/MHC class I) and costimulatory signals through CD80/86 and CD28. In addition, some CD8 T cell responses require additional signals from CD4 T cells and/or dendritic cells. It is thought that CD4 T cells provide signals to dendritic cells via CD40L-CD40 interactions to prime and form CTL memory. Another hypothesis is that CD4 cells stimulate CD8 T cells directly by binding to CD40 expressed on CD8 T cells (Grewal and Flavell 1998; Munroe, 2009). Both $CD40^{-/-}$ and $CD40L^{-/-}$ mice exhibit impaired *Chlamydia muridarum* infection clearance compared to WT mice, as well as increased susceptibility to *Leishmania* infection. Also, $CD40^{-/-}$ mice have increased susceptibility and mortality to West Nile virus infection. Accordingly, patients with HIGMI have increased susceptibility to *Toxoplasma gondii* infection and other opportunistic infections including *cryptosporidium*, *Pneumocystis carinii*, *mycobacteria*, and cytomegalovirus, underscoring the fact that cell-mediated immunity is jeopardized in these patients (Etzioni and Ochs 2004). Thus, CD40 signaling is required for effective pathogen clearance through the induction of an array of effector functions and at multiple stages of infection.

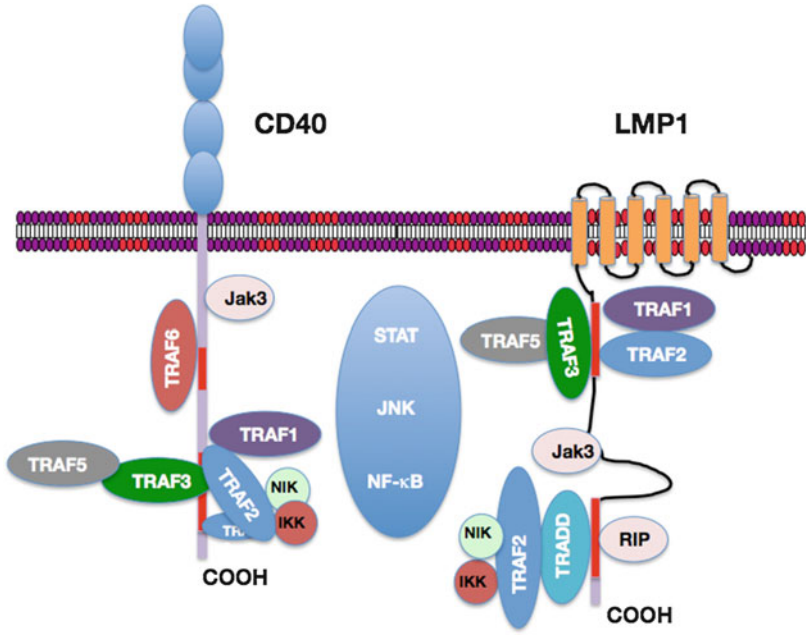
CD40 and Autoimmunity

The importance of CD40-CD40L interactions in mediating inflammatory responses formed the basis of investigating a possible role in autoimmune disorders. Not surprisingly, deregulated CD40-CD40L expression was found to be prominently featured in both systemic and tissue-specific autoimmunity (Peters et al. 2009). Importantly, anti-CD40L treatment was able to suppress autoantibody production in models of collagen-induced arthritis, systemic lupus erythematosus (SLE) nephritis, and experimental autoimmune encephalomyelitis (EAE). In addition, treatment of mice with anti-CD40L antibodies blocked the development of T cell-mediated disorders through interfering with the priming of self-recognizing T cells. In all cases, treatments were accompanied by a reduction in damage and/or infiltration of leukocytes to target tissues. Because many autoimmune disorders have a complex pathophysiology, the levels at which the blocking antibodies are acting are most likely highly variegated and include antigen presentation, antibody production, and induction of inflammatory cytokines (Peters et al. 2009). For both lupus nephritis and EAE, blocking antibodies interfered with ongoing disease, indicating that CD40-CD40L interactions are essential for the effector phase of disease.

In many autoimmune diseases, there is a significant increase in CD40 and/or CD40L expression; however, in most cases, heightened expression is not correlated with genetic polymorphisms at the respective loci. An exception to this observation is a CD40 polymorphism associated with the development of Graves' disease (GD). This disease leads to loss of thyroid gland function and is associated with autoantibodies against the thyroid-stimulating hormone receptor. In this particular case, linkage studies revealed that the CD40 gene locus was strongly linked with GD. This association is due to a C/T polymorphism in the Kozak consensus sequence that flanks the ATG start codon and is essential for translation initiation. Enhanced CD40 expression has been identified in thyroid tissue of GD patients bearing the C polymorphism and is thought to be a contributing factor to the pathogenesis of disease (Maier and Hafler 2009).

The Impact of CD40 on Atherosclerosis

Activated T cells and macrophages are important factors in the generation of inflammatory atherosclerotic plaques, and associated immune responses may, in some cases, constitute an autoimmune response against an unidentified antigen (Anand et al. 2003). Endothelial cells (EC), smooth muscle cells (SMC), and macrophage participate in atherogenesis (Anand et al. 2003). Importantly, human EC express CD40 and ligation through CD40L engagement leads to upregulation of adhesion molecules, which are



CD40, Fig. 5 A comparison of the intracellular domains of CD40 and LMP1 showing the approximate sites of TRAF binding as well as binding of Jak3, TRADD, RIP, NIK, and IKK. Listed within the *blue oval* are the pathways activated by those two molecules

also critical biomarkers of human atheromas. CD40 and CD40L are co-expressed on vascular endothelium and SMC in atherosclerotic lesions. Therefore, CD40L expressed by both the vascular wall and resident T cells is capable of ligating to CD40 expressed on EC, SMC, and infiltrating macrophage. This signaling cascade ultimately results in the expression of cytokines, matrix metalloproteinases, and adhesion molecules, all proteins normally present at high concentrations in human atheroma (Anand et al. 2003).

CD40 and Its Viral Mimic, LMP1

Epstein-Barr virus (EBV) is a γ-herpes virus that preferentially infects human B cells. Its successful persistence in cells is sustained by the function of several virally encoded proteins that are mimics of cellular factors critical for B cell physiology. The viral latent membrane protein 1 (LMP1) is one such protein and has been identified as the functional homologue of CD40 (Bishop and Hostager 2001). Important similarities exist between CD40 and LMP1 including the fact that signaling through either molecule leads to activation, proliferation, and survival of B cells. This functional identity reflects the ability of the intracellular domains of both LMP1 and CD40 to bind TRAF molecules and activate overlapping signaling pathways (Fig. 5). There are, however, recognized differences in the TRAF binding patterns of LMP1 and CD40 that suggest corresponding differences in select functional responses. Whereas both LMP1 and CD40 interact with TRAFs 1, 2, 3, and 5, only CD40 binds directly to TRAF6. Also, LMP1 requires TRAF3 as a positive regulator of NF-κB activity, whereas it is a negative regulator for the same pathways in B cells. Finally, LMP1 selectively binds to the tumor necrosis factor receptor-associated death domain (TRADD) protein and receptor-interacting protein (RIP) (Kilger et al. 1998).

Other distinctions in TRAF utilization directly reflect functional differences between CD40 and LMP1. As mentioned above, CD40L engagement with CD40 on B cells results in the association of CD40 with TRAF2/3 complexes; however, TRAF2 and 3 are rapidly ubiquitinated and degraded through the proteosome (Bishop et al. 2007). This degradation step leads to the downregulation of signal transmission. LMP1-mediated TRAF binding is highly stable and the difference in stability likely accounts for the fact that LMP1 provides a stronger and more

sustained signal than CD40. However, B cells that express both LMP1 and CD40 and receive signals through CD40-CD40L show an overall dampening of responses compared to LMP1 signaling alone. A plausible explanation for this lowered response is that selective TRAF degradation that accompanies CD40 signaling reduces the effective pool of TRAF3 available for subsequent signaling by LMP1. Another difference between CD40 and LMP1 is that LMP1 is constitutively located in the lipid rafts whereas CD40 moves into lipid rafts upon CD40L contact. Finally, the most striking difference between LMP1 and CD40 is that LMP1 constitutively signals in a ligand-independent manner through the self-aggregation of its 6 transmembrane-spanning domains and CD40 requires trimerization by binding to CD40L (Bishop and Hostager 2001).

To better define functional similarities of LMP1 and CD40 signaling, mice were created that expressed LMP1 in B cells deficient for CD40. Surprisingly, only a partial restoration of CD40 activity was achieved in inducing antibody class switching to IgG1, but not GC formation or the production of high-affinity antibodies after immunization. LMP1 expression even blocked GC formation in the presence of the endogenous CD40 receptor, indicating that LMP1 had an overall negative effect on differentiation. Also, constitutively active CD40 signaling in B cells produced by a fusion protein of the transmembrane domain of LMP1 and the signaling domain of CD40 inhibited the GC reaction, suggesting that GC formation can be blocked by the constitutive activation of B cells by either LMP1 or CD40 (Bishop et al. 2007).

CD40 and Cancer

The discovery that the CD40 pathway was absolutely critical for promoting B cell proliferation and rescue from apoptosis led to experiments that tested the role of CD40 in tumorigenesis. CD40 overexpression occurs in the vast majority of hematopoietic cancers and in greater than 75 % of all epithelial cancers. Also, in many cases, CD40 and CD40L are co-expressed on the same tumor cell. Accordingly, several hematopoietic cancers, including non-Hodgkin lymphoma, chronic lymphocytic leukemia, and Burkitt lymphoma, enhance proliferation and survival through an autocrine CD40-CD40L loop that acts primarily through the constitutive activation of NF-κB (Bereznaya and Chekhun 2007). This type of process may also be active in solid tumors that are capable of co-expressing CD40 and CD40L such as breast and kidney carcinoma and melanoma, where the presence of CD40L correlates with a more aggressive malignancy and shorter patient survival than tumors lacking CD40L (Bereznaya and Chekhun 2007). CD40 signaling can also drive tumor cell migration through the PI3K and NF-κB signaling pathways. It has been demonstrated that CD40 can indirectly promote tumor growth since chronically produced CD40L in the tumor microenvironment facilitates angiogenesis through the activation of CD40 expressed by endothelial cells. Metastasis can also be enhanced by tumor-expressing CD40 interacting with CD40L expressed on activated platelets (Bereznaya and Chekhun 2007).

It is clear that specific CD40-dependent signals are capable of providing growth and survival advantages to specific hematological malignancies; however, in a number of cases, CD40-CD40L interactions can also severely inhibit tumor growth. For example, a subset of aggressive B cell lymphomas respond to CD40 ligation with sensitization to apoptosis induced by chemotherapy, CD95/Fas engagement, or serum withdrawal. Also, CD40L treatment of primary high-grade B cell lymphoma, multiple myeloma, and Burkitt lymphoma results in a marked decrease in cell proliferation both in vitro and in xenotransplanted mouse models. Additionally, deregulation of the CD40 pathway has been shown to occur in CD4+ T cells from patients with chronic lymphocytic leukemia, and these leukemic cells are capable of suppressing CD40L expression in cocultured allogeneic T cells. In contrast, CD40 ligation of low-grade B cell malignancies, such as follicular lymphoma, chronic lymphocytic leukemia, and hairy cell leukemia, often stimulates cell

proliferation, underscoring the fact that the response to CD40 can be highly dependent on the differentiation state of the tumor.

The CD40 pathway is critical for the antitumor immune response through the function of DCs that are capable of cross-presenting tumor antigens to CD8 cytotoxic T cells and priming them for activation. Maturation of the DCs is required for effective priming, and this process is controlled by the binding of CD40 on the DCs with CD40L on CD4 T cells (Bereznaya and Chekhun 2007). Mature DCs have upregulated antigen-processing and presentation pathways and migrate to the lymph nodes to activate naïve CD4 and CD8 T cells. The requirement for CD40 in DC priming provides an explanation for the impaired tumor antigen-specific CTL activation in CD40-deficient mice. These findings confirm the requirement for CD40 in antitumor immunity and highlight the potential for this pathway being a viable target for cancer immunotherapy. Notably, multiple studies have addressed this and demonstrated that expression of CD40L in tumor cells or direct activation of DCs with anti-CD40 antibodies results in long-lasting systemic antitumor immune responses mediated by CD8 T cells.

Summary

CD40-CD40L interactions regulate the activation and the differentiation of a number of different cell types into effector cells. In particular, widespread expression of CD40 and CD40L has been demonstrated under pathological conditions, suggesting that this pathway is important for immune homeostasis and that deregulation leads to catastrophic consequences. Interference with CD40-CD40L interactions through the use of blocking antibodies reveals the potential of harnessing this receptor-ligand pair for the treatment of various autoimmune diseases and cancer. Finally, future work will more clearly elucidate the cross talk between the innate and adaptive immune responses and decipher how CD40-CD40L communication is moderated by signaling pathways initiated by viral infection and/or other types of immune challenges.

Cross-References

- ▶ Animal Models in Rheumatoid Arthritis
- ▶ B7 and CD28 Families
- ▶ Cytotoxic T Lymphocytes
- ▶ Immunodeficiency in Autoimmune Diseases
- ▶ Lymphocytes in Atherosclerosis
- ▶ NF-κB
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tumor-Infiltrating T Cells

References

Anand SX, Viles-Gonzalez JF, Badimon JJ, Cavusoglu E, Marmur JD. Membrane-associated CD40L and sCD40L in atherothrombotic disease. Thromb Haemost. 2003;90:377–84.

Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van Kooten C, Liu YJ, Rousset F, Saeland S. The CD40 antigen and its ligand. Annu Rev Immunol. 1994;12:881–922.

Benson MJ, Erickson LD, Gleeson MW, Noelle RJ. Affinity of antigen encounter and other early B-cell signals determine B-cell fate. Curr Opin Immunol. 2007;19:275–80.

Bereznaya NM, Chekhun VF. Expression of CD40 and CD40L on tumor cells: the role of their interaction and new approach to immunotherapy. Exp Oncol. 2007;29:2–12.

Bishop GA, Hostager BS. Signaling by CD40 and its mimics in B cell activation. Immunol Res. 2001;24:97–109.

Bishop GA, Xie P. Multiple roles of TRAF3 signaling in lymphocyte function. Immunol Res. 2007;39:22–32.

Bishop GA, Moore CR, Xie P, Stunz LL, Kraus ZJ. TRAF proteins in CD40 signaling. Adv Exp Med Biol. 2007;597:131–51.

Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev. 2009;229:152–72.

Etzioni A, Ochs HD. The hyper IgM syndrome–an evolving story. Pediatr Res. 2004;56:519–25.

Grewal IS, Flavell RA. The CD40 ligand. At the center of the immune universe? Immunol Res. 1997;16:59–70.

Grewal IS, Flavell RA. CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol. 1998;16:111–35.

Kilger E, Kieser A, Baumann M, Hammerschmidt W. Epstein-Barr virus-mediated B-cell proliferation is dependent upon latent membrane protein 1, which

simulates an activated CD40 recptor. EMBO J. 1998;17:1700–9.
Lederman S, Yellin MJ, Covey LR, Cleary AM, Callard R, Chess L. Non-antigen signals for B-cell growth and differentiation to antibody secretion. Curr Opin Immunol. 1993;5:439–44.
Loskog A, Totterman TH. CD40L – a multipotent molecule for tumor therapy. Endocr Metab Immune Disord Drug Targets. 2007;7:23–8.
Lougaris V, Badolato R, Ferrari S, Plebani A. Hyper immunoglobulin M syndrome due to CD40 deficiency: clinical, molecular, and immunological features. Immunol Rev. 2005;203:48–66.
Maier LM, Hafler DA. Autoimmunity risk alleles in costimulation pathways. Immunol Rev. 2009;229:322–36.
Munroe ME. Functional roles for T cell CD40 in infection and autoimmune disease: the role of CD40 in lymphocyte homeostasis. Semin Immunol. 2009;21:283–8.
Noelle RJ. The role of gp39 (CD40L) in immunity. Clin Immunol Immunopathol. 1995;76:S203–7.
Peters AL, Stunz LL, Bishop GA. CD40 and autoimmunity: the dark side of a great activator. Semin Immunol. 2009;21:293–300.
Tone M, Tone Y, Fairchild PJ, Wykes M, Waldmann H. Regulation of CD40 function by its isoforms generated through alternative splicing. Proc Natl Acad Sci USA. 2001;98:1751–6.
van Kooten C, Banchereau J. CD40-CD40 ligand. J Leukoc Biol. 2000;67:2–17.

CD5

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Synonyms

B reg – B regulatory cell; B10 – B cell producing IL-10; BAFF/BLyS – B cell activating factor/B lymphocyte stimulator BCR – B cell antigen receptor; CaMKII – Ca^{2+}/calmodulin-dependent kinase; CLL – chronic lymphocytic leukemia; DC – dendritic cell; EAE – autoimmune experimental acute encephalomyelitis; gp – glycoprotein; ITAM – Immunoreceptor Tyrosine-based Activation Motif; ITIM – Immunoreceptor Tyrosine-based Inhibitory Motif; MARCO – macrophage receptor with collagenous structure; MOG – myelin oligodendrocyte glycoprotein; PAMP – pathogen-associated molecular patterns; PC-PLC – Phosphatidylcholine-specific Phospholipase C; PI3K – Phosphatidylinositol 3-kinase; PRR – pathogen recognition receptor; PTP1C – Phosphotyrosine Phosphatase 1C; RAS-GAP-Ras GTPase activating protein; SHP-1 – Src homology region 2 domain-containing phosphatase-1; SLE – systemic lupus erythematosus; SRCR – scavenger receptor cysteine rich; T reg – regulatory T cell; TCR – T cell antigen receptor; TdT – terminal deoxynucleotidyl transferase

Definition

CD5 (previous names for CD5 have included Leu-1, Ly-1, OKT-1, T1, and Tp67) is a surface glycoprotein found in association with both the T cell antigen receptor (TCR) and the B cell antigen receptor (BCR). After the TCR or BCR is activated, CD5 is upregulated and undergoes intracellular phosphorylation of its cytoplasmic tail, although the precise role of CD5, before or after phosphorylation, is not yet clear. The 5′-flanking region of human *CD5* is transcriptionally active in T cells; the Ets transcription factors in conjunction with other regulatory elements are responsible for constitutive and tissue-specific CD5 expression (Arman et al. 2004). The levels of expression can be fine-tuned by both T cells and B cells (Soldevila et al. 2011).

There is evidence that CD5 transmits an inhibitory signal, suppressing or fine-tuning the T cell or B cell antigen-specific immune response. CD5 is also expressed on the surface of essentially all mature T cells and on thymocytes; before a monoclonal antibody to CD3 was developed, measurement of total CD5+ cell counts was often used to enumerate T cells. CD5 is present on more than 50 % of human fetal B cells but on only approximately 3 % of adult human lymph node B cells. Originally, the presence of CD5 on

B cells (albeit at about one-tenth the density as expressed on T cells) was used as the definition of a distinct B cell subpopulation, the B1 cell. The B1 cell appears early in fetal development; expresses CD5 and IgM, but little IgD; resides along pleural and peritoneal linings; is self-renewing, with a more limited repertoire (likely due to the absence of expression of terminal deoxynucleotidyl transferase [TdT]); and produces "natural antibodies." Natural antibodies (usually IgM but also IgG and IgA) show little somatic mutation; have low affinity for polysaccharides, as well as proteins and lipids; and often cross-react with self-antigens. A large proportion of the serum IgM in unimmunized mice comes from B1 cells. The B1 cell has been thought to represent a precursor population for auto-aggressive (autoimmune) B cell clones.

A population of peritoneal CD5 (−) B cells was identified whose surface phenotype was in all other ways similar to CD5+ B1 cells. The subsequent consensus terminology has been to designate CD5 (+) B1 cells as B1a cells and CD5 (−) B1 cells as B1b cells (Baumgarth 2011). Recently, CD5 has been identified on the transitional B cell population and on a newly described population of pre-naïve B cells (Lee et al. 2009).

CD5 is involved in the apoptosis of antigen-activated B cells and in the maintenance of tolerance by anergic B cells. CD5 cross-linking induces extracellular mobilization of calcium, tyrosine phosphorylation of a number of regulatory proteins at CD5 serine, threonine, and tyrosine residues, and the elaboration of diacylglycerol (Simarro et al. 1997).

The ligand(s) for CD5 have not yet been proven, but a large number of candidates have been identified.

Structure of CD5

CD5 is a glycoprotein of molecular mass 67kDa when reduced, 58kDa when not reduced. It is a member of the type B "scavenger receptor cysteine-rich" (SRCR) superfamily, an ancient and highly conserved group that includes CD6, MARCO (macrophage receptor with collagenous structure), Mac-2, M160 (CD163), M130, and a family of molecules expressed on γδ T cells, called WC (workshop cluster)-1. In humans, CD6 is only 82kb distant from CD5 on the long arm of chromosome 11 (11q13); the exon/intron organization of the human CD5 and CD6 genes is very similar, differing only in the size of intron 1 and the number of exons encoding their cytoplasmic regions; the conserved structure of the two loci, in both mouse and human, supports the premise that the two genes resulted from duplication of a primordial gene.

The common structure of members of the SRCR superfamily is a curved six-stranded β-sheet embracing an α-helix (Sarrias et al. 2004). These are transmembrane proteins with an extracellular region of three cysteine-rich scavenger receptor domains, each domain of approximately 100 amino acid residues encoded by a single exon. The cytoplasmic domain of about 90 amino acid residues is highly conserved and has no intrinsic enzymatic activity, although it contains many potential docking sites for secondary intracellular messengers, including activation molecules: Lck, PI3K (phosphatidylinositol 3-kinase), CaMKII (Ca^{2+}/calmodulin-dependent kinase), and PKC; negative regulators, C-Cbl, RAS-GAP (Ras GTPase-activating protein), and SHP-1 (Src homology region 2 domain-containing phosphatase-1); and the prosurvival molecules CK2 and Akt. Lck and RAS-GAP interact with CD5 via a pseudo-ITAM (immunoreceptor tyrosine-based activation motif) region found approximately in the middle of the intracytoplasmic region of CD5; CD5 also contains a membrane-proximal pseudo-ITIM (immunoreceptor tyrosine-based inhibitory motif) which may provide SHP-1 its docking site. CD5 also mediates activation of PC-PLC (phosphatidylcholine-specific phospholipase C), resulting in the production of diacylglycerol. In summary, CD5 is an accessory molecule whose cytoplasmic tail has no intrinsic catalytic activity but whose structures assist in activating a great many further signaling molecules.

There are two known isoforms of CD5, CD5-1a (membrane bound) and CD5-1b (intracellular form), differing in exon 1. (Please note that the two isoforms of CD5, CD5-1a and CD5-1b, have nothing to do with the two types of B1 cells, B1a and B1b.) The relative (and reciprocal) expression of the B1 isoforms is regulated by IL6 in patients with systemic lupus erythematosus (SLE), due to demethylation of the CD5-E1b promoter. This differential demethylation is more notable after BCR engagement, leading the authors to speculate that the loss of the inhibitory influence of CD5 might be involved in the immunopathogenesis of SLE (Garaud et al. 2009). The levels of these two forms are determined by regulating the levels of DNA methyltransferase with immunological consequences – decreased levels of expression of CD5-1a, the cell membrane form, are associated with lowering the threshold for BCR signaling. Defects in methyltransferase have been described in patients with SLE and in the MRL/lpr mouse which develops a lupus-like syndrome; there is recent evidence suggesting that microRNA abnormalities, specifically of mi-21 and mi-148a, may drive this defect in DNA methylation (Pan et al. 2010). Youinou and Renaudineau have discussed how such a defect in DNA methyltransferase activity could predispose to the development of SLE via decreased expression of CD5-1a (Youinou and Renaudineau 2011); this phenomenon is discussed below, under disease associations.

No alternative splicing forms have been reported.

Deletion mutations of the cytoplasmic region of CD5 have shown that retention of the single membrane-proximal tyrosine (within the pseudo-ITIM) was sufficient to maintain all observed negative regulatory activities and the ability to associate with SHP-1 (Berland and Wortis 2002).

There is also a soluble form of CD5 (sCD5), resulting from proteolytic cleavage of surface CD5 after cell activation. It is not clear if this form has regulatory or immunologic activity. Soluble CD5 is found at very low levels in the serum of normal individuals and in some patients with immune and other diseases, in some studies at higher levels. Patients with Sjogren syndrome were found to have elevated levels of sCD5, interpreted by the authors as evidence of lymphocyte activation (Ramos-Casals et al. 2001). Soldavila and colleagues speculate that the soluble form may act as a decoy receptor, which could interfere with CD5 functions as a survival mechanism and perhaps alter its regulatory capacity (Soldevila et al. 2011); a chimeric construct of murine CD5, called CD5Fc, led to apoptosis of activated T cells when given to Staphylococcus enterotoxin B-treated mice. As well, CD5Fc treatment induced the elimination of activated T cells and promoted recovery from experimental autoimmune encephalomyelitis (EAE); EAE in mice lacking CD5 was less severe and delayed than in control mice (Axtell et al. 2004). Vera and colleagues demonstrated that soluble human CD5 protects mice in a zymosan-induced model of septic shock (Vera et al. 2009). Thus, sCD5 may play a regulatory role in its own regard and may hold promise as a therapeutic in certain diseases of immune overstimulation, e.g., sepsis and autoimmune disorders.

Functions of CD5

CD5 associates with both the TCR and BCR, resulting in both quantitative and qualitative influences on signaling (Raman 2002). In its interaction with both, SHP-1 association with CD5 influences and downregulates the function of the antigen receptors; for the TCR, CD5-regulated substrates include CD3ξ, ZAP-70, Syk, and phospholipase Cγl but not the Src family tyrosine kinase p56 (lck) (Bondada et al. 2000, Perez-Villar et al. 1999). In thymocytes and B1a cells, CD5 provides inhibitory signals, whereas in peripheral mature T cells, it acts as a costimulatory signal receptor; thus, the CD5 signaling pathway can be either stimulatory or inhibitory, depending on circumstances, e.g., cell type, location, and stage of development (Lozano et al. 2000). Dalloul reviews the role of CD5+ B cells as regulatory B cells (Dalloul 2009).

Contrary to CD5 (−) B2 cells, B1 cells are long-lived because of autocrine expression of interleukin-10 (IL-10). Human peripheral B1 cells produce more IL-10 than do the far larger B2, CD5 (−) population following BCR activation. IL-10 production by B2 cells can be elicited after the introduction of CD5, by activation of the IL-10 promoter with mRNA synthesis. Only the cytoplasmic domain of CD5 is necessary for this effect. CD5 also protects normal human B cells from apoptosis after BCR stimulation. Thus, Gary-Gouy and colleagues showed that CD5 supports the survival of B cells by stimulating IL-10 production and by concurrently exerting negative feedback on BCR-induced signaling events that can promote antigen-stimulation induced cell death. (Gary-Gouy et al. 2002).

A unique population of CD5+ murine splenic cells, which are also CD1d+, expresses IL-10 and is thought to have regulatory roles in suppressing inflammation and autoimmune diseases (Watanabe et al. 2010); these are now called B10 cells and fall within a heterogenous population known as B reg (B regulatory cells), some of which are CD5-. B regs make up 1–2 % of splenic B cells and 7–8 % of peritoneal B cells but are not usually found in either lymph nodes or the peripheral blood. These cells may play a role in controlling a variety of autoimmune mouse models of disease, including SLE (Watanabe et al. 2010), collagen-induced arthritis, human rheumatoid arthritis (Mauri et al. 2003), contact dermatitis (Yanaba et al. 2008), and inflammatory bowel disease (Mizoguchi et al. 2002).

Lemoine et al. found that regulation of T cell responses was induced by B cells with the phenotype CD19(hi) IgD + CD38(hi) CD24(hi) CD5(hi) following CD40-dependent cognate interaction, and this regulation was indirect, being mediated by the induction of T regs, as indicated by the appearance of Foxp3 + CD4 + CD25 + T cells. T cell proliferation and cytokine production were differentially regulated. Thus, CD40-induced B regs partially inhibited T cell proliferation following CD40 interaction without the requirement for a soluble factor, whereas modulation of Th1 differentiation resulted from CD80/CD86-dependent interactions and from IL-10 production. Thus, activation signals from T cells appear to initiate regulatory properties in B cells that then, in turn, modulate T cell responses via the induction of T regs. Regulation of T cell proliferation was defective in SLE patients but efficient in other diseases, suggesting to the authors that this might be an active participant in the immunopathogenesis of autoimmune disease and a new therapeutic target (Lemoine et al. 2011).

Of note, Mizoguchi and colleagues subsequently found that the CD5 molecule has a role in colonic homeostatic immunomodulation. CD5 is expressed on most TCRγδ splenic T cells, whereas only a few intestinal intraepithelial TCRγδ T cells express CD5 in normal or TCRβ mutant $\beta^{-/-}$ mice; in the latter mutant mice, the absence of CD5 led to a remarkable increase in the CD4+ TCRγδ T cell population, also found to be increased in triple mutant MHC $II^{-/-}$ $CD5^{-/-}$ $\beta^{-/-}$ mice. CD4+ TCRγδ T cells provide help in Mycobacterium-induced germinal center formation and express a T_h-like cytokine profile. CD5+ TCRγδ T cells suppressed CD4+ TCR γδ T cell-mediated germinal center formation, thought by the authors to be due to the elimination of this CD4+ subset. CD5+ is expressed on >30 % of TCR γδ T cells in the colonic lamina propria, in contrast to the intraepithelial γδ T cell population. The absence of CD5 also led to increased numbers of CD4+ TCR γδ T cells in the colonic lamina propria and increased susceptibility to development of chronic colitis in $\beta^{-/-}$ mice. Cell transfer studies suggested that CD5+ TCR γδ T cells selectively eliminated CD4+ TCR γδ T cells in the intestine. The CD4+ TCR γδ T cells possess immune functions similar to CD4+ TCRαβ T cells (Mizoguchi, Mizoguchi et al. 2003).

In the EAE model of multiple sclerosis, B10 and classic T reg have different effects on disease initiation and persistence. Following inoculation with myelin oligodendrocyte glycoprotein (MOG) (33–35), B10 cell numbers expanded

quickly within the spleen but not in the central nervous system, and this expansion paralleled B10 cell regulation of disease initiation. Adoptive transfer of MOG (33–35)-sensitized B10 cells into wild-type mice suppressed the initiation of EAE but had no effect on established EAE, whereas Treg numbers expanded significantly within the CNS during progression and paralleled their ability to suppress established disease. Predictably, early depletion of B10 cells enhanced EAE severity, and Treg depletion enhanced late-phase disease (Matsushita et al. 2010). As noted above, EAE in mice lacking CD5 was less severe and was delayed in onset, compared with control mice (Axtell et al. 2004); thus, cells bearing CD5 may play a complex set of roles in the initiation and maintenance of EAE.

Murine CD5+ do not require BAFF/BLyS (B cell-activating factor/B lymphocyte stimulator) in contrast to their CD5 (−) brethren, which require this positive signaling molecule. However, BAFF can induce IL-10-producing B cells with a distinct CD1d(hi)CD5(+) phenotype, mainly derived from marginal zone B cells (Yanaba et al. 2008), via induction of activated transcription factor AP-1, which then binds to the IL-10 promoter; there is reason to believe that CD5 may be involved in the generation of B10 cells (Gary-Gouy et al. 2002). In murine studies, BAFF causes an increase in the number of IL-10-producing B cells in marginal zone regions. The BAFF-induced IL-10-producing B cells possess a regulatory function both in vitro and in vivo (Yang et al. 2010). Lo-Man recently reported that innate CD5(+) B regs negatively control innate inflammation and dendritic cell (DC) functions in neonatal mice by producing large amounts of IL-10 following Toll-like receptor triggering (Lo-Man 2011). CD5+ cells are also resistant to apoptosis due to Fas and membrane-bound immunoglobulin signaling, also explaining their prolonged survival.

CD5 antagonizes TCR activation by recruiting inhibitory intracellular mediators such as SHP-1, RAS-GAP, or Cbl, as noted previously. Bamberger and colleagues found that the inhibitory effects of CD5 also may occur via a parallel pathway through phosphorylation of the negative regulatory tyrosine (Tyr(531)) of Fyn, leading to significant reduction in Fyn kinase activity and inhibition of ZAP-70 activation. This effect requires the last 23 amino acids of the cytoplasmic domain of CD5, suggesting involvement of a new CD5-interacting signaling or adaptor protein. Of note, CD5 ligation leads to a shift in its membrane distribution from the bulk fluid phase to the lipid raft environment, where it forms homodimers and associates with Fyn, Lck, and PAG. The authors suggest this relocation may inhibit membrane-proximal signaling through control of phosphorylation and activity of Fyn, possibly by interfering with the disassembly of C-terminal Src kinase (Csk)-PAG-Fyn complexes during T cell activation (Bamberger et al. 2011).

CD5 can associate with CD2, CD3, CD4, CD8, and CD21 molecules; in thymocytes, CD5 can be found in association with PTP1C (phosphotyrosine phosphatase 1C), ZAP-70, p21 phospho-ξ, and PI3K, among other intracellular signaling molecules. As noted, T cells express higher levels of surface CD5 than B cells. CD5 is upregulated on T cells upon strong activation. In the thymus, there is a correlation between CD5 expression and the strength of the interaction of the developing T cell with self-peptides.

CD5 is prominently expressed on the surface of regulatory T cells (T regs), and Ordonez-Rueda and colleagues have suggested that CD5 regulates the generation and survival of natural T regs and furthermore provides a prosurvival signal in developing thymocytes; via phosphorylation of Akt, apoptosis is avoided (Ordoñez-Rueda et al. 2009). CD5 $^{-/-}$ have an enrichment of T reg in the thymus and the periphery as a result of a combination of deletion of naive thymocytes and increased generation of T reg due to increased TCR-mediated signaling. Expression of CD5 is apparently involved in modulating the function of CD4+ CD25+ T reg function, since Dasu and colleagues

demonstrated that these cells derived in $CD5^{-/-}$ mice are more effective in suppressing in vitro proliferation CD4+ CD25- responder T cells in response to anti-CD3 stimulation than were T regs from wild-type mice; this was not related to differences in intracellular expression of Foxp3. The severity of dextran sulfate sodium-induced colitis in $CD5^{-/-}$ mice was less than seen in wild-type mice (Dasu et al. 2008). Thus, there is evidence that CD5 is involved in immunomodulation, with a role in T reg function. Furthermore, both B cells (Hippens et al. 2000) and T cells (Stamou et al. 2003) that are unresponsive to antigen-specific stimulation have increased CD5 expression.

Further evidence of a role for CD5 in immune control is found in work by Hawiger and colleagues who identified a new mechanism for the induction of tolerance when they selectively targeted immature DC with the encephalitogenic self-antigen MOG to present this antigen to T cells in vivo. These DC resulted in peripheral T cell tolerance that interfered with induction of EAE. The tolerized T cells expressed elevated levels of CD5; this tolerance could not be invoked in $CD5^{-/-}$ mice. However, the tolerized cells were not truly anergic, since they could be activated by in vitro TCR stimulation. Thus, T cell tolerization involved the induction of CD5 on the affected cells (Hawiger et al. 2004).

There is also evidence that CD5 binds to zymosan, a β-glucan-rich particle expressed on fungi. Both membrane-bound and soluble CD5 bind to a variety of fungi, although not to bacterial components. This function is also served by several other SRCR proteins, e.g., CD6, MARCO, and CD163, although CD6 binds to bacterial antigens, e.g., lipopolysaccharide. Thus, CD5 acts as a pathogen recognition receptor (PRR) of pathogen-associated molecular patterns (PAMPs), i.e., a receptor that identifies "danger" and transmits the presence of pathogens to the innate immune system (Vera et al. 2009), e.g., PAMP recognition by PRRs activates DCs, enhancing their potency as antigen-presenting cells.

Potential CD5 Ligands

Many molecules have been claimed to be ligands for CD5, although none has yet to be universally accepted as such. These molecules include CD72 (a cell surface negative regulator of B cell activation from the pro-B through the mature B cell stage, also known as Lyb-2 in mice), pg35–37, gp77–80, gp150, gp200, the framework region of IgV_H (Soldevila et al. 2011), and CD5 itself (Brown and Lacey 2010). Zymosan, β-glycans, and fungi are capable of binding to all three extracellular domains of XCD5 (Soldevila et al. 2011).

Calvo and colleagues explored possible non-lymphocyte interactions of CD5 utilizing a soluble human CD5 extracellular domain glycoprotein (rsCD5). This molecule binds to monocytes, lymphocytes, and various cell lines of lymphoid, myelomonocytic, and epithelial origin, as do sCD5 and a human CD5-Ig fusion protein. The binding of rsCD5 may involve interactions of saccharides, since it is inhibited by high molar concentrations of certain monosaccharides (Calvo et al. 1999). Thus, CD5 may provide another means by which immune cells can communicate a state of immune activation to cells of a nonimmune lineage.

Disease Associations and Mechanisms Described for CD5

CD5 is a phenotypic marker for some B cell lymphoproliferative disorders, e.g., B cell chronic lymphocytic leukemia (CLL), mantle zone lymphoma, B cell non-Hodgkin's lymphoma, hairy cell leukemia, and small cell leukemia/lymphoma (Pangalis et al. 1999). About 76 % of T cell neoplasms express CD5. It is commonly lost in cutaneous T cell lymphoma; the absence of CD5 can be used as an indicator of degree of malignancy in this condition and serves as a poor prognostic factor for T cell acute lymphoblastic leukemia. Of note, CLL cells express the membrane form of CD5, which may provide them with a survival advantage (Soldevila et al. 2011).

The CD5+ B cell population (B1a cells) is expanded in some autoimmune disorders, including rheumatoid arthritis, Sjogren syndrome, insulin-dependent diabetes mellitus, and Graves' disease. The recently described CD5+ pre-naive (intermediate) B cell population is expanded in SLE patients and may be involved in the immunopathogenesis of autoimmunity (Lee et al. 2009). Hagn and colleagues found that serum levels of IL-21 and granzyme B (GzmB; a serine protease expressed by cytotoxic T lymphocytes and natural killer cells, which rapidly induces apoptosis of cells that on their surface bear "nonself" antigens, resulting from intracellular pathogens) correlated with each other strongly in patients with SLE. CD5+ B cells isolated from these patients constitutively express GzmB. IL-21 directly induced GzmB expression and in vitro secretion by these CD5+ B cells from lupus patients and patients with other autoimmune disease, as well as from cord blood. Coincident BCR activation caused a further increase in production of GzmB. Of note, IL-21 also suppressed both the viability and expansion of these lupus CD5+ B cells. Thus, CD5+ cells may be involved in the immunopathogenesis of SLE via a GZmB-related mechanism, and IL-21 may represent a viable therapeutic target (Hagn et al. 2010).

Initial studies implicated B1 cells as being the source of autoantibodies, but more recent work, reviewed by Youinou and Renaudineau, has found that B2 cells are actually the source of most high-affinity autoantibodies. Recent work suggests that, contrary to previous thinking, CD5+ B cells may actually play an important role in preventing autoimmunity, through its suppressive effects on BCR (and perhaps TCR) activation. The membrane expression of CD5 is regulated by the balanced expression of exon 1A (E1A) and exon 1B (E1B). The full-length protein variant, encoded by *E1A-cd5*, translocates the SHP-1 phosphatase to BCR and raises its activation threshold, thus controlling autoreactive B cell responses. However, the truncated variant, encoded *by E1B-cd5*, is not expressed on the cell surface; this intracytoplasmic CD5 traps SHP1. Under normal circumstances, EIB E1B is silenced by DNA methylation. Thus, defective DNA methylation prevents the effects of SHP-1, thereby favoring autoimmunity and perhaps the development of SLE (Youinou and Renaudineau 2011).

Conclusion

At least one of the roles CD5 plays may be modulation of immune responses, with the focus on suppression of autoimmune responses, but there is also the possibility that CD5+ functions may be dangerous, in that CD5 might enhance the survival of malignant T cells, allowing them to escape elimination. IL-10-producing B10 cells may also suppress immune responses to malignancies. Thus, CD5 is implicated in salubrious and pathological immunomodulation. There is a need for a better understanding of the factors that determine the number and potency of these cells in disease states, so that manipulation of the expression and function of CD5 can be used as a therapeutic intervention (Dalloul 2009).

As noted, the soluble form of CD5 may be implicated in immunomodulatory functions, suggesting one possible therapeutic strategy. Modification of function of the membrane-bound CD5 has been suggested as another means by which to either up- or downregulate immune function on T cells and B cells, as well as on the newly described subpopulation, B10 cells. Use of sCD5 may alter both autoimmune and malignant diseases and may be useful to block messages targeted to cells of an epithelial lineage, as noted above. The interaction of IL-21 with CD5+ cells may reveal other therapeutic targets.

Cross-References

- ▶ CTLA-4
- ▶ CTLA4-Ig
- ▶ Regulatory B Cells
- ▶ Resolution of Inflammation

▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
▶ Tregs in the Liver

References

Arman M, Calvo J, Trojanowska ME, Cockerill PN, Santana M, López-Cabrera M, Vives J, Lozano F. Transcriptional regulation of human CD5: important role of Ets transcription factors in CD5 expression in T cells. J Immunol. 2004;172:7519–29.

Axtell RC, Webb MS, Barnum SR, Raman C. Cutting edge: critical role for CD5 in experimental autoimmune encephalomyelitis: inhibition of engagement reverses disease in mice. J Immunol. 2004;173(5):2928–32.

Bamberger M, Santos AM, Gonçalves CM, Oliveira MI, James JR, Moreira A, Lozano F, Davis SJ, Carmo AM. A new pathway of CD5 glycoprotein-mediated T cell inhibition dependent on inhibitory phosphorylation of Fyn kinase. J Biol Chem. 2011;286(35):30324–36. Epub 2011 Jul 8.

Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective functions. Nat Rev Immunol. 2011;21(1):34–46.

Berland R, Wortis HH. Origins and functions of B-1 cells with notes on the role of CD5. Annu Rev Immunol. 2002;20:253–300. doi:10.1146/annurev.immunol.20.100301.064833.

Bondada S, Bikah G, Robertson DA, Sen G. Role of CD5 in growth regulation of B-1 cells. Curr Top Microbiol Immunol. 2000;252:141–9.

Brown MH, Lacey E. A ligand for CD5 Is CD5. J Immunol. 2010;185(10):6068–74.

Calvo J, Places L, Padilla O, Vilà JM, Vives J, Bowen MA, Lozano F. Interaction of recombinant and natural soluble CD5 forms with an alternative cell surface ligand. Eur J Immunol. 1999;29(7):2119–29.

Dalloul A. CD5: a safeguard against autoimmunity and a shield for cancer cells. Autoimmun Rev. 2009;8(4):349–53. Epub 2008 Nov 27.

Dasu T, Qualls JE, Tuna H, Raman C, Cohen DA, Bondada S. CD5 plays an inhibitory role in the suppressive function of murine CD4(+) CD25(+) T(reg) cells. Immunol Lett. 2008;119(1-2):103–13. Epub 2008 Jun 23.

Garaud S, Le Dantec C, Jousse-Joulin S, Hanrotel-Saliou C, Saraux A, Mageed RA, Youinou P, Renaudineau Y. IL-6 modulates CD5 expression in B cells from patients with lupus by regulating DNA methylation. J Immunol. 2009;182(9):5623–32.

Gary-Gouy H, Harriague J, Bismuth G, Platzer C, Schmitt C, Dalloul AH. Human CD5 promotes B-cell survival through stimulation of autocrine IL-10 production. Blood. 2002;100(13):4537–43. Epub 2002 Aug 8.

Hagn M, Ebel V, Sontheimer K, Schwesinger E, Lunov O, Beyer T, Fabricius D, Barth TF, Viardot A, Stilgenbauer S, Hepp J, Scharffetter-Kochanek K, Simmet T, Jahrsdörfer B. CD5+ B cells from individuals with systemic lupus erythematosus express granzyme B. Eur J Immunol. 2010;40(7):2060–9.

Hawiger D, Masilamani RF, Bettelli E, Kuchroo VK, Nussenzweig MC. Immunological unresponsiveness characterized by increased expression of CD5 on peripheral T cells induced by dendritic cells in vivo. Immunity. 2004;20(6):695–705.

Hippens KL, Tze LE, Behrens TW. CD5 maintains tolerance in anergic B cells. J Exp Med. 2000;191:883–90.

Lee J, Kuchen S, Fischer R, Chang S, Lipsky PE. Identification and characterization of a human CD5+ pre-naïve B cell population. J Immunol. 2009;182:4116–26.

Lemoine S, Morva A, Youinou P, Jamin C. Human T cells induce their own regulation through activation of B cells. J Autoimmun. 2011;36(3–4):228–38. Epub 2011 Feb 12.

Lo-Man R. Regulatory B, cells control dendritic cell functions. Immunotherapy. 2011;3(4 Suppl):19–20.

Lozano F, Simarro M, Calvo J, Vilà JM, Padilla O, Bowen MA, Campbell KS. CD5 signal transduction: positive or negative modulation of antigen receptor signaling. Crit Rev Immunol. 2000;20(4):347–58.

Matsushita T, Horikawa M, Iwata Y, Tedder TF. Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis. J Immunol. 2010;185(4):2240–52. Epub 2010 Jul 12.

Mauri C, Gray D, Mushtaq N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. J Exp Med. 2003;197(4):489–501.

Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. Immunity. 2002;16(2):219–30.

Mizoguchi A, Mizoguchi E, de Jong YP, Takedatsu H, Preffer FI, Terhorst C, Bhan AK. Role of the CD5 molecule on TCR gammadelta T cell-mediated immune functions: development of germinal centers and chronic intestinal inflammation. Int Immunol. 2003;15(1):97–108.

Ordoñez-Rueda D, Lozano F, Sarukhan A, Raman C, Garcia-Zepeda EA, Soldevila G. Increased numbers of thymic and peripheral CD4+ CD25 + Foxp3+ cells in the absence of CD5 signaling. Eur J Immunol. 2009;39(8):2233–47.

Pan W, Zhu S, Yuan M, Cui H, Wang L, Luo X, Li J, Zhou H, Tang Y, Shen N. MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. J Immunol. 2010;184(12):6773–81. Epub 2010 May 17.

Pangalis GA, Angelopoulou MK, Vassilakopoulos TP, Siakantaris MP, Kittas C. B-chronic lymphocytic leukemia, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma, including Waldenström's macroglobulinemia: a clinical, morphologic, and biologic spectrum of similar disorders. Semin Hematol. 1999;36(2):104–14.

Perez-Villar JJ, Whitney GS, Bowen MA, Hewgill DH, Aruffo AA, Kanner SB. CD5 negatively regulates the T-cell antigen receptor signal transduction pathway: involvement of SH2-containing phosphotyrosine phosphatase SHP-1. Mol Cell Biol. 1999;19(4): 2903–12.

Raman C. CD5, an important regulator of lymphocyte selection and immune tolerance. Immunol Res. 2002;26:255–63.

Ramos-Casals M, Font J, García-Carrasco M, Calvo J, Places L, Padilla O, Cervera R, Bowen MA, Lozano F, Ingelmo M. High circulating levels of soluble scavenger receptors (sCD5 and sCD6) in patients with primary Sjögren's syndrome. Rheumatology. 2001; 40(9):1056–9. doi:10.1093/rheumatology/40.9.1056.

Sarrias MR, Grønlund J, Padilla O, Madsen J, Holmskov U, Lozano F. The Scavenger Receptor Cysteine-Rich (SRCR) domain: an ancient and highly conserved protein module of the innate immune system. Crit Rev Immunol. 2004;24(1):1–37.

Simarro M, Pelassy C, Calvo J, Places L, Aussel C, Lozano F. The cytoplasmic domain of CD5 mediates both TCR/CD3-dependent and -independent diacylglycerol production. J Immunol. 1997;159(9):4307–15.

Soldevila G, Raman C, Lozano F. The immunomodulatory properties of the CD5 lymphocyte receptor in health and disease. Curr Opin Immunol. 2011;23:310–8.

Stamou P, de Jersey J, Carmignac D, Mamalaki C, Kioussis D, Stockinger B. Chronic exposure to low levels of antigen in the periphery causes reversible functional impairment correlating with changes in CD5 levels in monoclonal CD8 T cells. J Immunol. 2003;171(3):1278–84.

Vera J, Fenutría R, Cañadas O, Figueras M, Mota R, Sarrias MR, Williams DL, Casals C, Yelamos J, Lozano F. The CD5 ectodomain interacts with conserved fungal cell wall components and protects from zymosan-induced septic shock-like syndrome. Proc Natl Acad Sci USA. 2009;106(5):1506–11. Epub 2009 Jan 13.

Watanabe R, Ishiura N, Nakashima H, Kuwano Y, Okochi H, Tamaki K, Sato S, Tedder TF, Fujimoto M. Regulatory B cells (B10 cells) have a suppressive role in murine lupus: CD19 and B10 cell deficiency exacerbates systemic autoimmunity. J Immunol. 2010; 184(9):4801–9. Epub 2010 Apr 5.

Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF. A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. Immunity. 2008;28(5): 639–50.

Yang M, Sun L, Wang S, Ko KH, Xu H, Zheng BJ, Cao X, Lu L. Novel function of B cell-activating factor in the induction of IL-10-producing regulatory B cells. J Immunol. 2010;184(7):3321–5. Epub 2010 Mar 5.

Youinou P, Renaudineau Y. CD5 expression in B cells from patients with systemic lupus erythematosus. Crit Rev Immunol. 2011;31(1):31–42.

Cell Adhesion Molecules

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Synonyms

Cell adhesion molecules; Cell adhesion receptors

Definition

Cell adhesion molecules are involved in cell-cell and cell-matrix interactions and cell adhesion and migration in physiological processes and in diseases.

Introduction

Leukocytes, endothelial cells, and other cells, as well as communication of these cells by cell adhesion molecules (CAM) with each other and with the extracellular matrix, are highly involved in various physiological and immunopathological events. The most important adhesion molecules and their superfamilies will be introduced. Regarding the importance of CAMs in immunology and in clinics, this entry will concentrate on inflammation by presenting data on a prototype inflammatory disease, rheumatoid arthritis (RA).

The topics of natural immunity, tissue development, hemostasis, and other physiological conditions will not be discussed.

Adhesion Molecule Superfamilies

CAMs have been classified into integrin, selectin, immunoglobulin, and cadherin superfamilies (Springer 1990; Szekanecz et al. 1996; Agarwal and Brenner 2006).

Integrins

Integrins are αβ heterodimers. Each of the common β chains is associated with one or more α subunits (Albelda and Buck 1990; Springer 1990). Cell adhesion to the extracellular matrix (ECM) is mostly mediated by β_1 and β_3, while intercellular adhesion is facilitated through β_1 and β_2 integrins (Albelda and Buck 1990; Springer 1990; Imhof and Aurrand-Lions 2004). β_1 and β_3 integrins are expressed on endothelial cells, while β_2 integrins are leukocyte CAMs (Albelda and Buck 1990). The α_1–α_6, α_V, α_L, α_M, α_X, and β_1–β_7 integrin subunits have all been detected in the inflamed synovium (Johnson et al. 1993; Haskard 1995; Szekanecz et al. 1996; Agarwal and Brenner 2006). Only the most important integrins will be further discussed (Table 1).

Among β1 integrins, the α4β1 heterodimer, also known as very late antigen 4 (VLA-4) and clustered as CD49d/CD29, plays an important role in lymphocyte-endothelial interactions. This molecule recognizes at least two alternative ligands, such as vascular cell adhesion molecule 1 (VCAM-1), a member of the immunoglobulin superfamily of CAMs, and the CS-1 region of fibronectin. VLA-4 is present on lymphocytes and monocytes, while VCAM-1, an endothelial marker, can be induced by cytokines on a number of other cells (Albelda and Buck 1990; Springer 1990; Szekanecz et al. 1996). Regarding inflammation, the α4β1 integrin and VCAM-1 and CS-1 are expressed on various cell types within the RA synovium (Szekanecz et al. 1996; Agarwal and Brenner 2006).

Among other β_1 integrins, most members of theβ1 (VLA) family ($\alpha_{1\text{-}6}\beta_1$ dimers; CD49(a–f)/CD29) show increased expression on lymphocytes under inflammatory conditions (Johnson et al. 1993; Szekanecz et al. 1996; Agarwal and Brenner 2006). The predominant $CD4^+$ T lymphocyte subset is enriched in β_1 subunit positive ($CD29^+$) cells in the RA synovium. The expression of β_1 integrins is increased on extravascular compared to intravascular lymphocytes suggesting the upregulation of these receptors during extravasation into inflammatory sites (Johnson et al. 1993; Agarwal and Brenner 2006). Synovial lining cells also express the β_1 integrin subunit, while endothelial cells express $\alpha_1\beta_1$, $\alpha_3\beta_1$, and $\alpha_5\beta_1$ (Szekanecz et al. 1996).

All members of the β_2 (leukocyte) integrin family, namely, the lymphocyte function-associated antigen 1 (LFA-1) ($\alpha_L\beta_2$ dimer; CD11a/CD18), the Mac-1 ($\alpha_M\beta_2$; CD11b/CD18), and the p150,95 ($\alpha_X\beta_2$; CD11c/CD18) molecules, are present on neutrophils and monocytes. In contrast, only LFA-1 is present on lymphocytes. All three intercellular adhesion molecule (ICAM) ligands for LFA-1, namely, ICAM-1, ICAM-2, and ICAM-3, belong to the immunoglobulin superfamily. Mac-1 binds ICAM-1 and some non-CAM ligands. ICAM-1 is present on a number of cell types, while ICAM-2 and ICAM-3 are mostly expressed by endothelial cells and leukocytes, respectively (Albelda and Buck 1990; Springer 1990; Szekanecz et al. 1994a; Szekanecz et al. 1996). All β_2 integrins are expressed on synovial leukocytes (Haskard 1995; Szekanecz et al. 1996; Agarwal and Brenner 2006).

The β_3 integrins (gpIIb/IIIa molecule, $\alpha_{gpIIb}\beta_3$, CD41/CD61 and the vitronectin receptor, $\alpha_V\beta_3$, CD51/CD61) have been detected on macrophages, lining cells, and fibroblasts in the arthritic synovium (Johnson et al. 1993; Szekanecz et al. 1996). Among other integrins containing the α_V subunit, the fibronectin-vitronectin receptor $\alpha_V\beta_5$ is expressed by most, while $\alpha_V\beta_1$ and $\alpha_V\beta_3$ have been detected on some synovial lining cells. Their ligands, fibronectin, and vitronectin are also present in the arthritic synovial lining layer (Johnson et al. 1993; Szekanecz et al. 1996).

Another α_4 subunit-containing integrin, $\alpha_4\beta_7$, also binds to VCAM-1 and the CS-1 peptide

Cell Adhesion Molecules, Table 1 Some relevant adhesion receptor-ligand pairs in inflammation[a]

Adhesion receptors	Ligands
Integrins	
$\alpha_1\beta_1$ (VLA-1)	Laminin, collagen
$\alpha_2\beta_1$ (VLA-2)	Laminin, collagen
$\alpha_3\beta_1$ (VLA-3)	Laminin, collagen, fibronectin
$\alpha_4\beta_1$ (VLA-4)	Fibronectin, VCAM-1
$\alpha_5\beta_1$ (VLA-5)	Fibronectin
$\alpha_6\beta_1$ (VLA-6)	Laminin
$\alpha_L\beta_2$ (LFA-1, CD11a/CD18)	ICAM-1, ICAM-2, ICAM-3, JAM-A
$\alpha_M\beta_2$ (Mac-1, CD11b/CD18)	ICAM-2, iC3b
$\alpha_X\beta_2$ (CD11c/CD18)	iC3b, fibrinogen
$\alpha_E\beta_7$	E-cadherin
$\alpha_4\beta_7$	Fibronectin, VCAM-1, MadCAM-1
Immunoglobulin superfamily	
ICAM-1 (CD54)	LFA-1, Mac-1
ICAM-2	LFA-1
ICAM-3	LFA-1
VCAM-1	$\alpha_4\beta_1$, $\alpha_4\beta_7$
MadCAM-1	$\alpha_4\beta_7$, L-selectin
CD2	LFA-3
PECAM-1 (CD31)	PECAM-1, $\alpha_V\beta_3$
Selectins	
L-selectin (CD62L, LAM-1)	Sialylated carbohydrates, GlyCAM-1
E-selectin (CD62E, ELAM-1)	ESGL (sialyl-Lewis-X)
P-selectin (CD62P, PADGEM)	PSGL (sialyl-Lewis-X, other carbohydrates)
Cadherins	
E-cadherin (cadherin-1)	E-cadherin
N-cadherin (cadherin-2)	N-cadherin
Cadherin-11	Cadherin-11
Others	
CD44	Hyaluronic acid, fibronectin
Endoglin	TGF-β1, TGF-β3
JAMs	JAMs

[a]See text for abbreviations

(Albelda and Buck 1990). This integrin is present on more than 60 % of synovial lymphocytes. The $\alpha_4\beta_7$ integrin is highly expressed in tissues belonging to the mucosa-associated lymphoid tissue (MALT), such as the gut and skin, as well as the synovium. Therefore, this integrin may link arthritis to inflammatory bowel disease or psoriasis (Salmi et al. 1995).

The Immunoglobulin Superfamily of Adhesion Molecules

The immunoglobulin superfamily of CAMs is a group of transmembrane glycoproteins containing one or more immunoglobulin-like motifs of 60–100 amino acids (Albelda and Buck 1990; Springer 1990) (Table 1). VCAM-1, already mentioned above, is constitutively expressed on endothelial cells; however, its expression is upregulated by proinflammatory cytokines (Springer 1990; Szekanecz et al. 1996). ICAM-1, the counterreceptor for the β_2 integrins LFA-1 ($\alpha_L\beta_2$), Mac-1 ($\alpha_M\beta_2$), and $\alpha_X\beta_2$, is expressed on both endothelia and leukocytes (Albelda and Buck 1990; Springer 1990; Szekanecz et al. 1994a; Szekanecz et al. 1996). Among other ICAMs, ICAM-2 is constitutively expressed on endothelia

and may not be an activation marker (Szekanecz et al. 1994a). ICAM-3 is a leukocyte CAM; however, it is also present on some endothelial cells (Szekanecz et al. 1994a). All three ICAMs bind to β_2 integrins (Szekanecz et al. 1994a; Szekanecz et al. 1996). Other members of this superfamily include CD2 and LFA-3. These CAMs bind to each other (Albelda and Buck 1990; Springer 1990; Szekanecz et al. 1996). Platelet-endothelial adhesion molecule 1 (PECAM-1; CD31) mediates homotypic adhesion by binding to another PECAM-1 molecule, as well as heterotypic adhesion by recognizing the $\alpha_V\beta_3$ integrin (Springer 1990; Szekanecz et al. 1995; Szekanecz et al. 1996). PECAM-1 is a marker of endothelial activation (Springer 1990; Szekanecz et al. 1996).

As mentioned above, ICAMs serve as ligands for β_2 integrins. Under inflammatory conditions, ICAM-1 (CD54) is present on most endothelial cells, synovial lining cells, interstitial macrophages, lymphocytes, and synovial fibroblasts (Szekanecz et al. 1994a; Szekanecz et al. 1996; Agarwal and Brenner 2006). There is an increased ICAM-1 expression on HEV-like vessels in lymphocyte-rich areas compared to the "flat" endothelium of capillaries and venules in the RA synovium (Szekanecz et al. 1996). Reports showing that ICAM-2 (CD102) is equally expressed by most RA and normal synovial endothelia suggest that this CAM is not an activation antigen on endothelial cells (Szekanecz et al. 1994a). Upregulated expression of ICAM-3 (CD50) has been reported on cells of myeloid origin in the RA compared to normal synovial tissue. ICAM-3 could also be detected on about 10 % of RA synovial endothelial cells (Szekanecz et al. 1994a).

VCAM-1 is mentioned above in connection with the $\alpha_4\beta_1$ integrin. There is abundant VCAM-1 expression in the inflamed synovium, both on endothelial cells and leukocytes (Haskard 1995; Szekanecz et al. 1996; Agarwal and Brenner 2006).

Other CAMs in the immunoglobulin superfamily are expressed in inflammatory infiltrates. These adhesion pathways include the CD2/LFA-3 adhesion counterreceptors. While CD2 is a T cell marker, LFA-3 is present on endothelial and synovial lining cells, as well as macrophages and fibroblasts (Szekanecz et al. 1996; Agarwal and Brenner 2006).

PECAM-1 is abundantly expressed by most endothelial cells, and under inflammatory conditions, its expression is upregulated on synovial lining cells and interstitial macrophages (Johnson et al. 1993; Szekanecz et al. 1995; Szekanecz et al. 1996).

Interestingly, some sialylated and glycosylated CAMs, also known as tumor-associated antigens, such as members of the carcinoembryonic antigen (CD66) family also belong to the immunoglobulin superfamily. The CD66a-e molecules are structurally closely related to PECAM-1 and CD66 and, similarly to PECAM-1, exert abundant expression on myeloid cells in the inflamed synovium (Szekanecz et al. 1996; Szekanecz and Koch 2008).

Selectins

Selectins contain a lectin-like extracellular N-terminal domain, an epidermal growth factor (EGF)-like motif and two to nine moieties related to complement regulatory proteins (Albelda and Buck 1990; Patel et al. 2002) (Table 1). E-selectin and P-selectin are expressed by ECs, while L-selectin is mostly expressed by leukocytes (Patel et al. 2002). As described later, during leukocyte transendothelial migration, selectins mediate the initial tethering and rolling of leukocytes (Springer 1990; Butcher 1991; Patel et al. 2002). E-selectin is a marker for cytokine-induced endothelial activation (Patel et al. 2002). E-selectin ligand-1 (ESL-1) and P-selectin ligand-1 (PSGL-1) contain sialylated glycan motifs, such as sialyl Lewis-X (sLe^x) (Patel et al. 2002). P-selectin is constitutively present on the membrane of endothelial Weibel-Palade bodies (Patel et al. 2002). P-selectin is involved in the very early phases of leukocyte-endothelial adhesion (Butcher 1991). L-selectin serves as a lymphocyte homing receptor, where it mediates the physiological

recirculation of naive lymphocytes through specialized HEV (Springer 1990; Patel et al. 2002). However, L-selectin has also been implicated in inflammatory leukocyte recruitment (Szekanecz et al. 1996; Patel et al. 2002) (Table 1).

Selectins also play an important role in leukocyte adhesion to endothelium during the inflammatory process. There is increased endothelial expression of E-selectin (CD62E) in RA compared to non-inflamed synovial endothelial cells. E-selectin is a marker of endothelial activation in lymphocyte-rich areas. P-selectin (CD62P) is also present on arthritic synovial endothelia, while L-selectin (CD62L) is expressed by peripheral blood mononuclear cells. While other selectins can be induced upon endothelial activation, the cellular expression of L-selectin is decreased by shedding after cytokine treatment (Johnson et al. 1993; Johnson et al. 1993; Agarwal and Brenner 2006; Sarraj et al. 2006).

Cadherins

Cadherins are calcium-dependent CAMs. N-, P-, and E-cadherin are primarily involved in embryogenesis and tissue development; however, synovial fibroblast cadherin-11 has been implicated in arthritis as well (Albelda and Buck 1990; Springer 1990; Szekanecz et al. 1996; Agarwal and Brenner 2006) (Table 1).

Other Nonclassified Adhesion Receptors

The CD44 antigen has a proteoglycan-like structure; it is a receptor for hyaluronate and is expressed on activated ECs in inflammatory sites (Springer 1990; Johnson et al. 1993; Halloran et al. 1996; Szekanecz et al. 1996; Agarwal and Brenner 2006; Sarraj et al. 2006). CD44 is present on most synovial lymphocytes, as well as lining cells, leukocytes, endothelial cells, and fibroblasts. This CAM plays an important role in cellular adhesion, as well as activation in arthritis (Szekanecz et al. 1996; Agarwal and Brenner 2006; Sarraj et al. 2006) (Table 1).

Vascular adhesion protein 1 (VAP-1) was isolated from synovial endothelial cells. VAP-1 is a marker of activated endothelium, as its expression is increased in inflammation (Salmi et al. 1993).

Endoglin (CD105) has been identified as a receptor for transforming growth factor β1 TGF-β1 and TGF-β3. This molecule contains the RGD (arginine-glycine-aspartate) sequence, a motif found in a number of extracellular matrix ligands for integrins. Thus, endoglin may be involved in intercellular adhesion. Endoglin was detected on most inflamed and normal endothelial cells. In arthritis, endoglin expression is increased on synovial lining cells and macrophages (Szekanecz et al. 1996; Szekanecz and Koch 2008).

Junctional cell adhesion molecules (JAMs) are also involved in transendothelial migration of leukocytes. JAM-A and JAM-C have been implicated in adhesive events underlying inflammatory conditions, such as RA (Imhof and Aurrand-Lions 2004; Rabquer et al. 2008).

The Role of Adhesion Molecules in Leukocyte Extravasation to Inflammatory Sites

Leukocyte extravasation into the synovium and into other inflammatory sites is an active process mediated by a number of CAMs (reviewed in Jalkanen 1989; Albelda and Buck 1990; Springer 1990; Haskard 1995; Szekanecz et al. 1996; Agarwal and Brenner 2006). The inflammatory cascade of leukocyte adhesion and migration begins with the adhesion of neutrophils and mononuclear cells to endothelium (Butcher 1991; Ziff 1991; Szekanecz et al. 1996). Inflammatory vessels, such as the specialized synovial venules resembling morphology similar to high endothelial venules (HEV), are present in the arthritic synovium (Szekanecz et al. 1996; Agarwal and Brenner 2006). Leukocytes transmigrate through the vessel wall, and then they adhere to other cells in the synovium, such as synovial lining cells, interstitial macrophages, and fibroblasts (Jalkanen 1989;

Szekanecz et al. 1996; Agarwal and Brenner 2006). Thus, leukocyte ingress into the inflamed synovium may be considered as pathological leukocyte "homing" into the joints, which is a temporally and sequentially well-regulated process.

The *temporal* organization of leukocyte migration into the joint is difficult to assess in humans. In animal models, such as rat adjuvant-induced arthritis (AA), neutrophils are present in the synovium approximately 1-week post-adjuvant injection, followed by the ingress of lymphocytes and monocyte/macrophages after another 4–6 days. These events are accompanied by the temporally regulated expression of various CAMs including selectins, integrins, and CD44 (Halloran, Szekanecz et al. 1996).

The *sequential* regulation of the adhesion cascade is similar in various types of inflamed tissues. The initial adhesion of leukocytes to endothelium ("tethering" and "rolling") is mediated mainly by selectins. This is followed by leukocyte activation, as well as the upregulation and conformational changes in the expression of a number of CAMs, mainly leukocyte (β2) integrins, resulting in firm, more stable interactions. Several CAMs including integrins and CD31 may be involved in the transendothelial migration of leukocytes into the synovium; however, some aspects of these molecular mechanisms are not fully understood (Haskard 1995; Szekanecz et al. 1996; Agarwal and Brenner 2006).

The relationship between CAM expression and the preferential "homing" of lymphocytes into certain tissues (e.g., lymph nodes, spleen, MALT) is still poorly understood. No CAM is exclusively responsible for leukocyte migration into the synovium or other tissues. However, as described below, functional human and animal studies suggest that some adhesion pathways may be more important in the synovium than others (Haskard 1995; Szekanecz et al. 1996; Imhof and Aurrand-Lions 2004; Agarwal and Brenner 2006). The tissue specificity of leukocyte migration may be important for the explanation of organ involvement in various inflammatory diseases. For example, gut-derived, mucosal T cells and immunoblasts express CAM profiles characteristic for memory cells. These cells bind well to synovial, as well as gut HEV. In addition, the α4β7 integrin described above is highly expressed on both synovial and gut lymphocytes, suggesting the role of this integrin in the pathogenesis of arthritis associated with inflammatory bowel diseases. However, mucosal lymphocyte adhesion to synovial endothelium utilizes mainly α4β1-, while that to gut HEV mostly α4β7-dependent mechanisms, showing the involvement of various CAM-mediated pathways within the MALT. In general, the adhesive mechanism of lymphocyte "homing" into the synovium is similar to that into MALT rather than that into peripheral lymph nodes (Salmi et al. 1995).

Assessment of Inflammatory Cell Adhesion in Experimental Systems

The functional role of CAMs and the interactions of these molecules with inflammatory mediators have been investigated in a number of experimental systems. Cell cultures of isolated endothelial cells or fibroblasts are suitable for studies on CAM expression regulation by cytokines, while the relative importance of certain adhesion pathways can be determined by using in vitro adhesion assays. Animal models are used to characterize all these mechanisms in vivo.

Fibroblast cultures express both ICAM-1 and VCAM-1. Among proinflammatory cytokines, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-4, and interferon-γ (IFN-γ) stimulate the adhesion of leukocytes to fibroblasts. Furthermore, IL-1β, TNF-α, IFN-γ, basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and granulocyte-monocyte colony-stimulating factor (GM-CSF) may induce ICAM-1, while IL-1β, IL-4, TNF-α, and IFN-γ may stimulate VCAM-1 expression on fibroblasts. Some cytokines may also stimulate LFA-3 expression on these cells (Szekanecz et al. 1996).

In endothelial cultures, ICAM-1 expression can be induced by IL-1β, TNF-α, and IFN-γ. IL-1β stimulates endothelial E-selectin

expression as well. Peripheral blood monocytes obtained from RA patients adhere well to both resting and cytokine-stimulated synovial endothelial cells, accompanied by increased monocyte β2 integrin expression (Haskard 1995; Szekanecz et al. 1996).

In vitro adhesion assays utilize leukocytes layered on endothelial cell and fibroblast cultures and frozen tissue sections ("frozen section assay") as well as culture dishes coated with extracellular matrix components. Blocking studies may be performed by introducing anti-CAM monoclonal antibodies (mAb) to these assays. The degree of adhesion inhibition reflects the relative importance of the studied CAMs in intercellular adhesion. Furthermore, as cell cultures can be activated with inflammatory mediators, cell-cytokine-CAM interactions can be investigated. For example, blocking studies using mAbs to β1 and β2 integrins clarified the role of adhesion pathways mediated by these integrins in arthritis. In the "frozen section" assay, blocking studies have shown the importance of β1 (mainly α4β1), β2 (mainly LFA-1), and α4β7 integrins; P-, E-, and L-selectins; as well as CD44 in lymphocyte adhesion to synovial HEV-like venules. Adhesion studies using dishes coated with fibronectin revealed the functional role of integrins in the adhesion of synovial lymphocytes to the extracellular matrix. T cell-fibronectin adhesion could be blocked by mAbs to α4β1 and α5β1 integrins, as well as by their respective ligands, LDV- and RGD-containing fibronectin peptides (Jalkanen 1989; Haskard 1995; Salmi et al. 1995; Agarwal and Brenner 2006).

Some aspects of CAM expression and CAM-cytokine interactions in arthritis are difficult to assess in human systems. There are a number of in vivo animal models of arthritis available. Apart from the classical rat arthritis induced by adjuvant (AIA), additional models include streptococcal wall-, type II collagen- (CIA), and proteoglycan-induced arthritis (PGIA) (Halloran, Szekanecz et al. 1996; Szekanecz et al. 1996; Sarraj et al. 2006). For example, in rat AIA, β2 integrins were detected on myeloid cells, while ICAM-1 was expressed on most endothelial cells in the arthritic synovium. CD44 also exerts variable expression in the arthritic synovium in AIA and PGIA (Halloran et al. 1996; Szekanecz et al. 1996; Sarraj et al. 2006).

Adhesion Molecules in Atherosclerosis

Atherosclerosis may be considered as an inflammatory disease (Ross 1993). In addition, accelerated atherosclerosis and increased vascular damage, as well as increased cardio- and cerebrovascular morbidity and mortality, have been associated with arthritides (Gonzalez-Gay et al. 2006; Szekanecz et al. 2007). Several adhesion molecules, including integrins, VCAM-1, ICAM-1, ICAM-3, and selectins, have been implicated in the pathogenesis of inflammatory atherosclerosis (Ross 1993; Gonzalez-Gay et al. 2006; Szekanecz et al. 2007) as well as in the pathogenesis of inflammatory aortic aneurysms (Szekanecz et al. 1994b). Furthermore, anti-TNF biologics may suppress adhesion molecule expression in the vessel wall (Gonzalez-Gay et al. 2006).

Blockade of Adhesion Molecules: A Yet Unfulfilled Dream in Anti-Inflammatory Therapy

Certainly, corticosteroids and other immunosuppressive drugs may have modulatory effects on CAM expression and inflammatory cell adhesion (Szekanecz et al. 1996; Agarwal and Brenner 2006; Gonzalez-Gay et al. 2006); however, these nonspecific therapies will not be further discussed.

Regarding specific anti-CAM targeting in humans, first an antihuman ICAM-1 antibody (enlimomab) was used to treat refractory RA. Many patients reported improvement in their status; however, repeated administration of this antibody resulted in diminished efficacy and frequent adverse events. Therefore, further development of enlimomab in RA was terminated (Szekanecz et al. 1996). Two anti-integrin

strategies, the anti-LFA-1 antibody efalizumab and the LFA-3-Ig fusion protein alefacept, have been registered for the treatment of psoriasis. Alefacept also yielded to a moderate effect in psoriatic arthritis (Menter 2009). Efalizumab has recently been withdrawn from the market due to severe side effects. Natalizumab (anti-α_4 integrin) has been registered for the treatment of multiple sclerosis and Crohn's disease. Various other anti-integrin and anti-CD44 antibodies have been tried in animal models of arthritis, but not yet in human disease (Szekanecz et al. 1996; Agarwal and Brenner 2006; Sarraj et al. 2006). The high number of CAMs and the redundancy of adhesion network and multiple regulatory pathways may account for the notion that targeting of one CAM may be technically feasible, but it often yields to disappointing results in human trials.

Cross-References

- ▶ Chemokines
- ▶ Mechanisms of Endothelial Activation

References

Agarwal SK, Brenner MB. Role of adhesion molecules in synovial inflammation. Curr Opin Rheumatol. 2006;18(3):268–76.

Albelda SM, Buck CA. Integrins and other cell adhesion molecules. FASEB J. 1990;4(11):2868–80.

Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell. 1991;67(6):1033–6.

Gonzalez-Gay MA, Garcia-Unzueta MT, et al. Influence of anti-TNF-alpha infliximab therapy on adhesion molecules associated with atherogenesis in patients with rheumatoid arthritis. Clin Exp Rheumatol. 2006;24(4):373–9.

Halloran MM, Szekanecz Z, et al. Cellular adhesion molecules in rat adjuvant arthritis. Arthritis Rheum. 1996;39(5):810–19.

Haskard DO. Cell adhesion molecules in rheumatoid arthritis. Curr Opin Rheumatol. 1995;7(3):229–34.

Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. Nat Rev Immunol. 2004;4(6):432–44.

Jalkanen S. Leukocyte-endothelial cell interaction and the control of leukocyte migration into inflamed synovium. Springer Semin Immunopathol. 1989;11(2):187–98.

Johnson BA, Haines GK, et al. Adhesion molecule expression in human synovial tissue. Arthritis Rheum. 1993;36(2):137–46.

Menter A. The status of biologic therapies in the treatment of moderate to severe psoriasis. Cutis. 2009;84(Suppl 4):14–24.

Patel KD, Cuvelier SL, et al. Selectins: critical mediators of leukocyte recruitment. Semin Immunol. 2002;14(2):73–81.

Rabquer BJ, Pakozdi A, et al. Junctional adhesion molecule C mediates leukocyte adhesion to rheumatoid arthritis synovium. Arthritis Rheum. 2008;58(10):3020–9.

Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993;362(6423):801–9.

Salmi M, Andrew DP, et al. Dual binding capacity of mucosal immunoblasts to mucosal and synovial endothelium in humans: dissection of the molecular mechanisms. J Exp Med. 1995;181(1):137–49.

Salmi M, Kalimo K, et al. Induction and function of vascular adhesion protein-1 at sites of inflammation. J Exp Med. 1993;178(6):2255–60.

Sarraj B, Ludanyi K, et al. Expression of CD44 and L-selectin in the innate immune system is required for severe joint inflammation in the proteoglycan-induced murine model of rheumatoid arthritis. J Immunol. 2006;177(3):1932–40.

Springer TA. Adhesion receptors of the immune system. Nature. 1990;346(6283):425–34.

Szekanecz Z, Haines GK, et al. Increased synovial expression of the adhesion molecules CD66a, CD66b, and CD31 in rheumatoid and osteoarthritis. Clin Immunol Immunopathol. 1995;76(2):180–6.

Szekanecz Z, Haines GK, et al. Differential distribution of intercellular adhesion molecules (ICAM-1, ICAM-2, and ICAM-3) and the MS-1 antigen in normal and diseased human synovia. Their possible pathogenetic and clinical significance in rheumatoid arthritis. Arthritis Rheum. 1994a;37(2):221–31.

Szekanecz Z, Kerekes G, et al. Accelerated atherosclerosis in rheumatoid arthritis. Ann N Y Acad Sci. 2007;1108:349–58.

Szekanecz Z, Koch AE. Vascular involvement in rheumatic diseases: 'vascular rheumatology'. Arthritis Res Ther. 2008;10(5):224.

Szekanecz Z, Shah MR, et al. Intercellular adhesion molecule-1 (ICAM-1) expression and soluble ICAM-1 (sICAM-1) production by cytokine-activated human aortic endothelial cells: a possible role for ICAM-1 and sICAM-1 in atherosclerotic aortic aneurysms. Clin Exp Immunol. 1994b;98(2):337–43.

Szekanecz Z, Szegedi G, et al. Cellular adhesion molecules in rheumatoid arthritis: regulation by cytokines and possible clinical importance. J Investig Med. 1996;44(4):124–35.

Ziff M. Role of the endothelium in chronic inflammatory synovitis. Arthritis Rheum. 1991;34(11):1345–52.

Chemokines

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Synonyms

Chemoattractant; Chemotactic proteins; Small cytokines

Chemokines: A General Definition

Chemokines are a large family of small chemotactic cytokines involved in many physiological processes, including development, angiogenesis, tissue organization, cell homing, and inflammation. Most of these functions are associated with cellular migration and directional movement in response to specific chemokine gradients. Chemokines are also important in cellular activation and cellular protection against damage. Chemokine signaling is mediated through interactions with 7-transmembrane, G protein-coupled receptors that subsequently results in the diverse functions described above. Dysregulation of chemokine secretion directly participates in the pathogenesis of many diseases, by altering inflammation, cell survival, homeostasis, resolution of injury, migration of stem or hematopoietic cells, cellular differentiation, and scar formation as well as by global regulation of the immune response. Thus, chemokines and their receptors participate in normal physiology as well as in pathologic conditions in all tissues.

Classification: Chemokines are divided into four subfamilies according to the position of two conserved cysteine (C) residues in their amino (N)-terminus: CC ligands (CCL), CXCL, CX3CL, and XC (X represents a different amino acid). Each chemokine activates specific chemokine receptors (CCR) (see summary of chemokines and their receptors in Table 1) that, depending upon the cell type that expresses the receptor, results in diverse functions, including migration, synaptic plasticity, or regeneration. Chemokine receptors belong to the superfamily of G protein-coupled receptors (GPCRs) (Onuffer and Horuk 2002). These receptors are 7-transmembrane proteins and signal mainly through G proteins. Chemokines have different target cells according to the expression of their chemokine receptors; however, in addition to this variable, tissue localization and kinetics of expression during development or inflammation also play key roles in the function of these chemokines. Their expression can be induced by various stimuli, including growth factors, inflammatory mediators, and/or cellular debris in normal and pathologic conditions (Onuffer and Horuk 2002).

Role of Chemokines During Inflammation

During inflammation, leukocyte recruitment occurs from the bone marrow and blood into the injured/inflamed tissues. This leukocyte recruitment involves leukocyte rolling, firm adhesion, and subsequent diapedesis of cells into affected tissues in response to specific chemokine gradients and the interaction of the chemokines with the appropriate chemokine receptors on the surface of the leukocytes. Most of the chemokines released during inflammation are synthesized de novo; however, some chemokines are constitutively expressed at low levels, and some are intracellularly stored in granules, for example, in endothelial cells, platelets, and cytotoxic T cells, perhaps to mediate a rapid response (Celie et al. 2009). Some of these stored chemokines include CCL1, CCL5, CCL7, CXCL1, CXCL4, CXCL5, and CXCL8. Chemokines also interact with the extracellular matrix and remain there for a long period of time attached to cells (Imberty et al. 2007; Witt and Lander 1994), including endothelial cells,

Chemokines, Table 1 Chemokines and their receptors

CC family		
New nomenclature	Old nomenclature	Receptor
CCL1	I-309	CCR8
CCL2	MCP-1, monocyte chemotactic protein-1	CCR2/CCR4
CCL3	MIP-1α, macrophage inflammatory protein-1α	CCR1/CCR5
CCL4	MIP-1β, macrophage inflammatory protein-1β	CCR5
CCL5	RANTES, regulated upon activation, normal T cells expressed and secreted	CCR5
CCL6	–	CCR1/?
CCL7	MCP-3, monocyte chemotactic protein-3	CCR1/CCR2
CCL8	MCP-2, monocyte chemotactic protein-2	CCR2/CCR3
CCL9	MIP-1β, macrophage inflammatory protein-1γ or macrophage inflammatory protein-related protein-1 (MRP-2) and CCF18; also named CCL10 (not used any more)	?
CCL10	Now is named CCL9	–
CCL11	Eotaxin-1	CCR3
CCL12	MCP-5 or Scya12	CCR2?
CCL13	MCP-4, Scya13	CCR2/CCR3
CCL14	HCC-1	CCR1
CCL15	Leukotactin-1, MIP-5, and HCC-2	CCR1/CCR3
CCL16	HCC-4 and Scya16	CCR1/CCR2/CCR5/ CCR8
CCL17	TARC	CCR4
CCL18	PARC, pulmonary and activation-regulated chemokine	CCR6
CCL19	EBl1 ligand chemokine (ELC) or macrophage inflammatory protein-3-beta (MIP-3β)	CCR7
CCL20	Macrophage inflammatory protein-3 (MIP-3A)	CCR6
CCL21	Secondary lymphoid-tissue chemokine (SLC), 6Ckine (because it has 6 conserved cysteine residues instead of 4 as other chemokines) and exodus-2	CCR7
CCL22	Scya22	CCR4
CCL23	MIP-3 and myeloid progenitor inhibitory factor 1 (MPIF-1)	CCR1/?
CCL24	Myeloid progenitor inhibitory factor 2 (MPIF-2) or eotaxin-2	CCR3/?
CCL25	TECK (thymus-expressed chemokine)	CCR9
CCL26	Eotaxin-3, MIP-4α, and thymic stroma chemokine-1	CCR3
CCL27	Cutaneous T cell-attracting chemokine (CTACK)	CCR10
CCL28	Mucosae-associated epithelial chemokine (MEC)	CCR3/CCR10
CCL29	–	Unknown
CXC family		
New nomenclature	Old nomenclature	Receptor
CXCL1	Growth-regulated protein alpha (GRO1 or α) oncogene and neutrophil-activating protein 3 (NAP-3)	CXCR2 > CXCR1
CXCL2	GROβ	CXCR2
CXCL3	GROγ and MIP2β	CXCR2
CXCL4	Platelet factor 4 (PF4)	Splice variant of CXCR3, CXCR3B
CXCL5	Neutrophil-activating peptide 78 (ENA-78)	CXCR2
CXCL6	Granulocyte chemotactic protein 2 (GCP-2)	CXCR1/CXCR2
CXCL7	NAP-2	CXCR2
CXCL8	Interleukin-8 (IL-8)	CXCR1/CXCR2

(*continued*)

Chemokines, Table 1 (continued)

CXC family		
New nomenclature	Old nomenclature	Receptor
CXCL9	Monokine induced by gamma interferon (MIG)	CXCR3
CXCL10	Interferon gamma-induced protein 10 (IP-10) or small inducible cytokine B10	CXCR3
CXCL11	Interferon-inducible T cell alpha chemoattractant (I-TAC) or IP-9	CXCR3
CXCL12	Stromal cell-derived factor-1 (SDF-1a)	CXCR4
CXCL13	B lymphocyte chemoattractant (BCA-1)	CXCR5
CXCL14	Breast- and kidney-expressed chemokine (BRAK)	?
CXCL15	Lungkine	?
CXCL16	Scyb16	CXCR6
CXCL17	Scyb17	?
C family		
New nomenclature	Old nomenclature	Receptor
XCL1	Lymphotactin	XCR1
XCL2	Scyc2	XCR1
CX_3C family		
New nomenclature	Old nomenclature	Receptor
CX3CL1	Fractalkine	CX3CR1

?: unknown or unclear

generating microenvironments that control baseline cell homing and trafficking, or cell repulsion, termed fugetaxis (Poznansky et al. 2002; Vianello et al. 2005).

Binding of a chemokine to its receptor on the surface of a leukocyte results in activation of integrins by a $G_{\alpha i}$ protein-dependent mechanism, leading to calcium flux and activation of phosphatidylinositol 3-kinase (PI3K) and Rho GTPase pathways (Mellado et al. 2001a). This signaling facilitates cytoskeletal changes in the leukocyte, resulting in formation of cellular processes, polarization, and directional movement towards higher concentrations of chemokines. However, high concentrations of chemokines can also induce a repulsive signal (Poznansky et al. 2002; Vianello et al. 2005), though the signaling and mechanisms that mediate these diametric differences are not yet well understood.

The ability of chemokine receptors to dimerize and heterodimerize results in even more complex chemokine-chemokine receptor signaling (Mellado et al. 2001b). The aggregation of chemokine receptors, similar to cytokine receptors, can result in different signaling according to the kinetics and type of receptor activated. Activation of monomeric chemokine receptors ($<$15 s) results in activation of phospholipase, calcium, and PI3K. Activation of oligomer chemokine receptors (20–60 s) results in kinase activity (PKB, ERK, Src, and JAK), and clustering of these receptors ($>$60 s) results in arrestin-mediated internalization and desentization (Delgado et al. 2001; Thelen 2001). Chemokine receptor signaling induced by binding of specific chemokine(s) is extremely transient. This is because G_{α} subunits, after activation to hydrolyze GTP, rapidly recombine with $G_{\beta\gamma}$ subunits to form the inactive conformation that was present before chemokine activation. Additionally, receptor desensitization occurs, especially by receptor phosphorylation by G protein-coupled receptor kinases (GRK). Receptor downregulation also occurs caused by β-arrestin or adaptin-2-mediated receptor sequestration and intracellular internalization through clathrin-coated pits or caveolae and regulated by receptor phosphorylation as well (see review by Rot and von Andrian 2004). Interestingly, in lymph node endothelial cells, the protein chemokine interceptor D6 targets many CC chemokines for internalization and subsequent degradation (Rot 2010), but the role of this protein in other tissues or during disease processes has not been

examined further. The contribution of each mechanism to chemokine signaling and chemoattraction continues to be studied.

In normal and inflammatory conditions, leukocytes are chemoattracted to a specific tissue in response to chemokine gradients. First, tethering occurs to establish a weak interaction between leukocytes and the endothelium, in part mediated by selectins. The next step is rolling that slows down leukocytes and establishes a stronger EC-leukocyte interaction, partially mediated by selectins and integrins. The third step is conformational changes in the integrins and upregulation of adhesion proteins on the endothelial cell and leukocyte, resulting in a firm adhesion that is mediated by cell adhesion molecules (CAM) including ICAM-1 and VCAM-1 expressed on the endothelium and integrins VLA-4 and LFA-1 on the leukocyte (▶ Cell Adhesion Molecules). These critical interactions mediate tight adhesion and subsequent directed intravascular diapedesis between or through endothelial cells into the target tissue (see review by Roberts et al. 2010). During the normal process of diapedesis, leukocytes migrate across the endothelium by specific interactions between adhesion molecules, tight junction proteins, integrins, cytoskeletal proteins and activation of metalloproteinases, chemokines, and chemokine receptors. The coordination of these interactions results in a controlled transmigration of leukocytes across the endothelium into target tissues, with no significant changes in permeability. However, in inflammatory conditions, this controlled transmigration is dysregulated and changes in endothelial permeability and increased numbers of leukocytes transmigrating occur. Thus, changes in any component of the transmigration process result in development of several human diseases, including autoimmune processes, developmental abnormalities, cancer, and inflammation.

There are many examples of the role of chemokines in normal and pathologic conditions. Only a few will be discussed in this review to highlight the varied functions of chemokines.

Chemokines and HIV: Currently there are an estimated 33 million people infected with HIV. Despite advances in prevention, treatment, and survival, there is still a significant percentage of the population living with the virus and with HIV-related illness. HIV infects mainly cells expressing CD4 and CCR5 or CXCR4. The chemokine receptors CCR5 and CXCR4 are co-receptors for the virus, enabling HIV entry into susceptible cells. This entry into $CD4^{+}CCR5^{+}$ T cells results in ~50 % of the cells being infected and destroyed in the first 2–3 weeks postinfection (Mattapallil et al. 2005). However, other cell types such as monocyte/macrophages, dendritic cells, and a specific naïve T cell population maintain the virus without toxic consequences, establishing reservoirs that harbor the virus for long periods of time (Lafeuillade and Stevenson 2011).

The ligands for CCR5, CCL3 (MIP-1α), CCL4 (MIP-1β), or CCL5 (RANTES) are produced by many cell types, including macrophages, T cells, dendritic cells, γδ T cells, NK cells, and astrocytes, and prevent HIV and simian immunodeficiency virus (SIV) entry by competing for binding to the chemokine receptor and by downregulating surface expression of these receptors (Cocchi et al. 1995; Pinto et al. 2000). The critical role of chemokine receptors in HIV/AIDS is underscored by the finding that individuals expressing the polymorphisms for CCR5Δ32 are protected from HIV infection (Reiche et al. 2007). Compounds that block the ability of the HIV envelope to bind to chemokine receptors to reduce viral entry have been developed, and these therapies are currently in use (Choi and An 2011). However, reducing or blocking these receptors may have other consequences. For example, CXCL12 or CXCR4 knockdown mice die during embryogenesis with several hematopoietic, gastrointestinal, cardiac, vascular, and brain defects (Nagasawa et al. 1996; Zou et al. 1998). In the brain of these knockout mice, the cerebellum, hippocampal dentate gyrus, cortex, and dorsal root ganglia are defective in migration and proliferation of progenitor cells (Dziembowska et al. 2005). Thus, some concerns related to the long-term use of CXCR4-blocking agents exist, especially as HIV-infected individuals live longer.

In addition to the role of chemokine receptors in HIV entry, chemokine and chemokine receptor expression also regulate the pathogenesis of HIV disease. Data in NeuroAIDS indicates that HIV infection of leukocytes upregulates the chemokine receptor CCR2 (Eugenin et al. 2006), which facilitates the transmigration of high numbers of HIV-infected leukocytes across a tissue culture model of the blood-brain barrier (Eugenin et al. 2006). The higher expression of this chemokine receptor on HIV-infected leukocytes may enable these cells to sense lower amounts of CCL2 from the brain parenchyma and initiate their transmigration into the brain (Eugenin et al. 2006). The mechanism by which HIV regulates the expression and function of these chemokine receptors requires further investigation.

In addition, other viruses, such as pox and herpes viruses, encode chemokines and chemokine receptors in their genomes that become expressed after infection of different cells. This expression has been proposed to participate in viral replication, migration, and cellular proliferation and to attract new circulating cells to amplify infection (Lalani et al. 2000; Murphy et al. 2001). Thus, chemokine and chemokine receptors, in addition to protecting from injury, can also be hijacked by different viruses to spread infection, evade an immune response, and induce inflammation and cellular damage.

Chemokines and Cancer: Chemokines play a key role in cancer. As discussed above, the action of chemokines and their chemokine receptors is dependent in part upon the organ in which they are expressed and where the chemokine(s) is released. Metastatic cancer cells migrate to other tissues through the blood, and several chemokines and chemokine receptors have been identified that participate in this process. Expression of CXCR4 and CCR7 in breast cancer cells has been associated with metastasis to the lung and lymph node (Cabioglu et al. 2005). Melanoma cells expressing CXCR4 and CCR7 are associated with pulmonary and lymph node metastasis (Takeuchi et al. 2004). High expression of CXCR4 has been described as a prognostic marker of myelogenous leukemia, and low expression is associated with a better prognosis (Kalinkovich et al. 2006). An example of the critical role of chemokines and their receptors in cancer has been demonstrated in pancreatic cancer, where CD133+/CXCR4+ tumor cells at the edge of the tumor are the cells that determine the metastatic capacity without affecting the growth of the primary tumor (Hermann et al. 2007). In agreement, mammary tumor cells that expressed higher amounts of CXCR4 correlate with the aggressiveness of the tumor. Inhibition of the CXCR4/CXCL12 axis using compounds that block interaction with CXCR4, such as AMD3100, reduces chemotaxis of tumor cells in vitro and suppresses tumor angiogenesis and tumor growth in vivo (Liang et al. 2007). This compound is being used in ongoing clinical trials related to metastasis (Lazennec and Richmond 2010).

Although we briefly discussed data for a role of CXCR4 and CCR7 in the pathogenesis of cancer, other receptors and chemokines are also involved. CXCL1, 2, 3, 5, 6, 8, 10, and 12; CCL2, 3, and 4 and their receptors; CXCR1, 2, 4, 5, 6, 7, 9, and 10; and CCR1, 2, 4, 5, 6, 7, 9, and 10 are also involved in different stages of the malignant process (see details in Lazennec and Richmond 2010). Changes in expression of these receptors and chemokines suggest that tissue-specific chemokine gradients enable cancer cells to colonize different tissues (▶ Tumor-Infiltrating T Cells).

Chemokines in Lymphocyte Homing and Trafficking

Lymphocytes, during their development and specialization, traverse long distances, from the bone marrow and/or thymus, circulating in the blood, homing to secondary lymphoid organs. All these processes of migration, selection, and trafficking into different tissues are controlled by chemokines. In addition, chemokines regulate integrin activation that results in intracellular signaling and cell adhesion (Kim et al. 2004). Many of these effects for T cells occur in the thymus. The adult thymus can be divided into the central medulla and the peripheral cortex. The cells present in these areas can be divided into hematopoietic cells from bone marrow

origin, such as thymocytes, dendritic cells, NK cells, and macrophages; stromal cells; and mesenchymal cells. The cortex is composed of high numbers of immature thymocytes supported by a complex network of thymic epithelial cells and dendritic cells. In contrast, the medulla has fewer thymocytes, at an immature stage, supported by thymic epithelial cells and dendritic cells (see details in Dzhagalov and Phee 2012).

Thymocytes are normally in constant motion due to their patterns of differentiation, proliferation, and selection. Chemoattraction, repulsion, and adhesion to stromal cells are essential to these processes and are mediated mostly by chemokines expressed by different cell types and in different locations within the thymus. Some of the cells that regulate these changes in movement of thymocytes are dendritic cells, endothelial cells, and epithelial cells. Different chemokine gradients, including those for CCL25, CCL21, CCL19, CCL17, and CXCL12, recruit different subsets of thymocytes (see details in Dzhagalov and Phee 2012; Le Borgne et al. 2009). Thus, alterations in expression of these chemokines or their receptors result in significant changes in differentiation and selection of T cells, leading to immune deficiencies or autoimmune diseases.

Some of these critical steps mentioned above have been demonstrated by examining lymphocyte trafficking and cell-to-cell interactions required for immune activation and migration that are dependent on CCL21 and CCR7. Expression of CCR7 is increased in a subset of thymocytes following positive selection, and the ligands for CCR7 are expressed mostly in the medulla, suggesting that selected thymocytes are preferentially recruited to the medulla by a mechanism that involves CCR7 (Kurobe et al. 2006). In agreement, CCR7 transgenic thymocytes showed an increased frequency of maturation (Kurobe et al. 2006). In addition, in other tissues, CCL21 or CCR7 genetic depletion reduced migration of T cells into lymph nodes (Cyster et al. 1999) (see details of chemokine-chemokine receptors in Table 1). CXCR5 and CCR7 are essential for migration of B cells from T cell-rich zones into B cell follicles in the spleen or the Peyer's patches (Forster et al. 1999; Gatto et al. 2011). Thus, specific changes in chemokine-chemokine receptor expression in a site-specific manner control migration, differentiation, selection, and egress of subsets of lymphocytes, suggesting that any alterations in these processes mediated by chemokines and chemokine receptors could result in immunodeficiency and/or autoimmune disease.

Conclusion

Chemokines and their receptors are essential for normal development, cellular homing, immune surveillance, regeneration, and cellular and tissue plasticity, as well as for inflammation, cellular damage, and disease. These ligand-receptor interactions are mainly mediated by the specific localization of the ligand (chemokine) and its receptor on different cells and tissues. These interactions, ligand-receptor, are tightly controlled; however, during disease pathogenesis, dysregulation of either one results in exacerbation or lack of resolution of injury. In addition, some infectious agents, such as HIV, use specific chemokine receptors to enable entry into susceptible cells, which suggests that pathogens evolved to use this important communication system to survive within a host. Other pathogens also alter the secretion of chemokines to impair and evade immune or inflammatory responses. Understanding the regulation of chemokines and their receptors should enable the design of more effective therapies to target disease processes.

References

Cabioglu N, Sahin A, Doucet M, Yavuz E, Igci A, Yildirim EO, Aktas E, Bilgic S, Kiran B, Deniz G, et al. Chemokine receptor CXCR4 expression in breast cancer as a potential predictive marker of isolated tumor cells in bone marrow. Clin Exp Metastasis. 2005;22:39–46.

Celie JW, Beelen RH, van den Born J. Heparan sulfate proteoglycans in extravasation: assisting leukocyte guidance. Front Biosci. 2009;14:4932–49.

Choi WT, An J. Biology and clinical relevance of chemokines and chemokine receptors CXCR4 and CCR5 in human diseases. Exp Biol Med (Maywood). 2011;236:637–47.

Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science. 1995;270:1811–5.

Cyster JG, Ngo VN, Ekland EH, Gunn MD, Sedgwick JD, Ansel KM. Chemokines and B-cell homing to follicles. Curr Top Microbiol Immunol. 1999;246:87–92. discussion 93.

Delgado MB, Clark-Lewis I, Loetscher P, Langen H, Thelen M, Baggiolini M, Wolf M. Rapid inactivation of stromal cell-derived factor-1 by cathepsin G associated with lymphocytes. Eur J Immunol. 2001;31:699–707.

Dzhagalov I, Phee H. How to find your way through the thymus: a practical guide for aspiring T cells. Cell Mol Life Sci. 2012;69:663–82.

Dziembowska M, Tham TN, Lau P, Vitry S, Lazarini F, Dubois-Dalcq M. A role for CXCR4 signaling in survival and migration of neural and oligodendrocyte precursors. Glia. 2005;50:258–69.

Eugenin EA, Osiecki K, Lopez L, Goldstein H, Calderon TM, Berman JW. CCL2/monocyte chemoattractant protein-1 mediates enhanced transmigration of human immunodeficiency virus (HIV)-infected leukocytes across the blood-brain barrier: a potential mechanism of HIV-CNS invasion and NeuroAIDS. J Neurosci. 2006;26:1098–106.

Forster R, Schubel A, Breitfeld D, Kremmer E, Renner-Muller I, Wolf E, Lipp M. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell. 1999;99:23–33.

Gatto D, Wood K, Brink R. EBI2 operates independently of but in cooperation with CXCR5 and CCR7 to direct B cell migration and organization in follicles and the germinal center. J Immunol. 2011;187:4621–8.

Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell. 2007;1:313–23.

Imberty A, Lortat-Jacob H, Perez S. Structural view of glycosaminoglycan-protein interactions. Carbohydr Res. 2007;342:430–9.

Kalinkovich A, Tavor S, Avigdor A, Kahn J, Brill A, Petit I, Goichberg P, Tesio M, Netzer N, Naparstek E, et al. Functional CXCR4-expressing microparticles and SDF-1 correlate with circulating acute myelogenous leukemia cells. Cancer Res. 2006;66:11013–20.

Kim M, Carman CV, Yang W, Salas A, Springer TA. The primacy of affinity over clustering in regulation of adhesiveness of the integrin {alpha}L{beta}2. J Cell Biol. 2004;167:1241–53.

Kurobe H, Liu C, Ueno T, Saito F, Ohigashi I, Seach N, Arakaki R, Hayashi Y, Kitagawa T, Lipp M, et al. CCR7-dependent cortex-to-medulla migration of positively selected thymocytes is essential for establishing central tolerance. Immunity. 2006;24:165–77.

Lafeuillade A, Stevenson M. The search for a cure for persistent HIV reservoirs. AIDS Rev. 2011;13:63–6.

Lalani AS, Barrett JW, McFadden G. Modulating chemokines: more lessons from viruses. Immunol Today. 2000;21:100–6.

Lazennec G, Richmond A. Chemokines and chemokine receptors: new insights into cancer-related inflammation. Trends Mol Med. 2010;16:133–44.

Le Borgne M, Ladi E, Dzhagalov I, Herzmark P, Liao YF, Chakraborty AK, Robey EA. The impact of negative selection on thymocyte migration in the medulla. Nat Immunol. 2009;10:823–30.

Liang Z, Brooks J, Willard M, Liang K, Yoon Y, Kang S, Shim H. CXCR4/CXCL12 axis promotes VEGF-mediated tumor angiogenesis through Akt signaling pathway. Biochem Biophys Res Commun. 2007;359:716–22.

Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature. 2005;434:1093–7.

Mellado M, Rodriguez-Frade JM, Manes S, Martinez AC. Chemokine signaling and functional responses: the role of receptor dimerization and TK pathway activation. Annu Rev Immunol. 2001a;19:397–421.

Mellado M, Vila-Coro AJ, Martinez C, Rodriguez-Frade JM. Receptor dimerization: a key step in chemokine signaling. Cell Mol Biol (Noisy-le-Grand). 2001b;47:575–82.

Murphy AM, Sheahan BJ, Atkins GJ. Induction of apoptosis in BCL-2-expressing rat prostate cancer cells using the Semliki Forest virus vector. Int J Cancer. 2001;94:572–8.

Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature. 1996;382:635–8.

Onuffer JJ, Horuk R. Chemokines, chemokine receptors and small-molecule antagonists: recent developments. Trends Pharmacol Sci. 2002;23:459–67.

Pinto LA, Williams MS, Dolan MJ, Henkart PA, Shearer GM. Beta-chemokines inhibit activation-induced death of lymphocytes from HIV-infected individuals. Eur J Immunol. 2000;30:2048–55.

Poznansky MC, Olszak IT, Evans RH, Wang Z, Foxall RB, Olson DP, Weibrecht K, Luster AD, Scadden DT. Thymocyte emigration is mediated by active movement away from stroma-derived factors. J Clin Invest. 2002;109:1101–10.

Reiche EM, Bonametti AM, Voltarelli JC, Morimoto HK, Watanabe MA. Genetic polymorphisms in the chemokine and chemokine receptors: impact on clinical course and therapy of the human immunodeficiency virus type 1 infection (HIV-1). Curr Med Chem. 2007;14:1325–34.

Roberts TK, Buckner CM, Berman JW. Leukocyte transmigration across the blood-brain barrier: perspectives on neuroAIDS. Front Biosci. 2010;15:478–536.

Rot A. Chemokine patterning by glycosaminoglycans and interceptors. Front Biosci. 2010;15:645–60.
Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annu Rev Immunol. 2004;22:891–928.
Takeuchi H, Fujimoto A, Tanaka M, Yamano T, Hsueh E, Hoon DS. CCL21 chemokine regulates chemokine receptor CCR7 bearing malignant melanoma cells. Clin Cancer Res. 2004;10:2351–8.
Thelen M. Dancing to the tune of chemokines. Nat Immunol. 2001;2:129–34.
Vianello F, Olszak IT, Poznansky MC. Fugetaxis: active movement of leukocytes away from a chemokinetic agent. J Mol Med (Berl). 2005;83:752–63.
Witt DP, Lander AD. Differential binding of chemokines to glycosaminoglycan subpopulations. Curr Biol. 1994;4:394–400.
Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature. 1998;393:595–9.

Complement in Rheumatic Diseases

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Synonyms

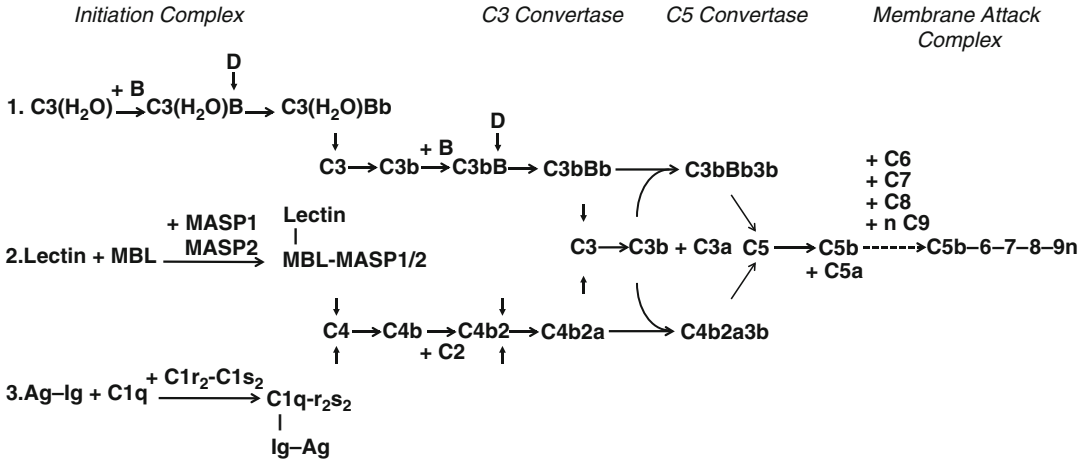
B, Bf, CFB, complement factor B; C4A, Rodger blood group antigen, complement C4A; C4B, Chido blood group antigen, complement C4B; C5aR, CD88, complement C5a receptor; C5L2, GPR77, alternative complement C5a receptor; cC1qR, calreticulin, receptor for the collagen stalk of C1q; CR1, CD35, complement receptor type 1; CR2, CD21, complement receptor type 2; CR3, CD11b, ITGAM, complement receptor type 3, alpha chain; CR4, CD11c, ITGAX, complement receptor type 4, alpha chain; D, CFD, complement factor D, adipsin; DAF, CD55, decay-accelerating factor; gC1qR, C1qbp, receptor for the globular head of C1q; H, CFH, complement factor H; I, CFI, complement factor I; MCP, CD46, membrane cofactor protein; RP1, STK19; RP2, STK19P, serine/threonine kinase 19 gene and pseudogene

Definition

The complement system is a primary immune effector mechanism for the humoral immune response. The complement system consists of effector proteins, regulators, and receptors. There are three pathways that trigger cascades of activation through proteolysis of zymogens present in the circulation. These pathways converge at the activation of complement C3 and C5 and progress to the formation of the membrane attack complexes (MACs) on target membranes. Anaphylatoxins C3a and C5a are generated during the activation process. The cascades of complement system are regulated and adjusted according to the type of initiators, and the microenvironment in which complement activation is occurring. Proteins of the complement system cooperate and coordinate to differentiate among invading microbes, immune complexes, apoptotic cells, cellular debris, and physiologic host cells (Law and Reid 1995; Ricklin et al. 2010; Walport 2001a, b).

Pathways of Complement Activation

Three distinct pathways are engaged in the initiation of complement activation (Fig. 1). The classical pathway was discovered first,



Complement in Rheumatic Diseases, Fig. 1 The three activation pathways of the complement system, drawn according to evolution and physiologic sequences. Pathway 1 is known as the alternative pathway. It is activated through a tick-over mechanism because of continuous hydrolysis of the thioester bond in C3, which enables the formation with factor B, a weak C3 convertase. Pathway 2 is known as the MBL or lectin pathway. It is initiated through the binding of mannan-binding lectin (MBL) or ficolin to arrays of simple sugar molecules in glycosylated antigens on microbes. This is a pattern recognition mechanism characteristic of the innate immune system. Pathway 3 is initiated through the binding of specific antibodies IgM or IgG to antigens. It is an effector arm of the humoral adaptive immune system. Each activation pathway engages the formation of a multi-molecular initiation complex, followed by the assembly of a C3 convertase and a C5 convertase for activations of C3 and C5, respectively, and culminates in the formation of membrane attack complexes (MACs) on the target membrane. Anaphylatoxins C3a and C5a are produced during the activation process. For brevity, by-products generated during the activation of C4, C2, and factor B are not shown. *Vertical arrows* show activation of component proteins through cleavage by serine proteinases. A *dotted horizontal arrow* denotes multiple steps are involved in the formation of the membrane attack complex

historically, but it is the youngest in evolutionary terms. It is a key effector for the humoral adaptive immune response. In 1895, Jules Bordet demonstrated the presence of an inborn and heat-labile serum factor that was necessary to assist or "complement" antibodies in killing bacterial targets. It is now known that such inborn "complement factor" consists of more than 50 proteins. Nine different complement components named as numerals C1 to C9 – in the order of discovery – exist as zymogens, or inactive, mature secretory proteins in the blood circulation. The formation of antibody-antigen complexes exposes binding sites for C1q on Fc-regions of immunoglobulins, triggering the assembly and activation of the multi-molecular C1 complex, C1q-$C1r_2$-$C1s_2$ (Pathway 3, Fig. 1). Activated C1s in the C1 complex is a serine proteinase, which activates C4 and C2. The larger products of the enzyme digests, C4b and C2a, respectively, assemble to form the classical pathway C3 convertase (abbreviated C4b2a). The C4b subunit binds to the activator surface covalently through the carbonyl group of the glutamine-994 residue (Q994) from its newly exposed thioester bond after activation. The C2a subunit (in the C3 convertase) is a serine proteinase that cleaves C3 to C3a and C3b. Similar to C4b, C3b also consists of a highly reactive thioester bond that binds covalently to nearby membranes, or else it is hydrolyzed. The association of C3b with C4b2a changes the multi-molecular enzyme to the C5 convertase (abbreviated C4b2a3b). The C5 convertase cleaves C5 to C5a and C5b. C5b binds to target membranes through hydrophobic interaction, exposing binding sites for C6 and then C7. The C5b-7 complex then inserts to the lipid bilayers of the cell membrane and attracts the binding of C8. C5b-7 and C5b-8 complexes can partially lyse the target cells (i.e., are sub-lytic), thereby causing leakage of cytoplasm. On microbial cell

surfaces, C5b-8 initiate polymerization of multiple molecules of C9, forming membrane attack complexes (MACs). The MAC creates pores in the target membranes, which induces cell lysis, loss of cytoplasm, and osmotic shock.

During the activation process, two potent anaphylatoxins are generated by C3 and C5 convertases, C3a and C5a, respectively (Zhou 2012). Interactions of C3a and/or C5a with their corresponding receptors on granulocytes stimulate discharge of granules that have vasoactive, toxic, and bacteriocidal properties. In addition, C5a is a strong chemoattractant. It stimulates migrations of neutrophils to the site of complement activation. Thus, complement activation is linked to inflammatory response.

In the mid-1950s, Pillemer observed that complement activation could occur in the absence of a specific antibody. The existence of such an "*alternative*" pathway of activation was challenged, but confirmed more than two decades later. Specific protein factors involved in this alternative pathway are named in alphabets, such as factor B, factor D, factor H, and P (properdin). This pathway is initiated by a "tick-over" mechanism, by which a small proportion of complement C3 in the circulation is constantly hydrolyzed at slow rate by water to form $C3(H_2O)$. $C3(H_2O)$ may weakly associate with factor B. Serine proteinase factor D cleaves factor B, releasing Ba from the complex. $C3(H_2O)Bb$ is a weak C3 convertase that cleaves C3 to C3b and C3a (Pathway 1, Fig. 1). Most C3b generated in this way is inactivated by hydrolysis of the internal thioester bond. However, if the C3b is attached to a microbial or "protected" surface, factor B will associate with it and become activated by factor D, forming C3bBb. C3bBb is a powerful alternative pathway C3 convertase, which activates C3 to generate C3b and C3a. The binding of properdin to C3bBb on a microbial (or protected) surface will further stabilize the convertase and enhance its activity. The freshly produced C3b molecules associate with factor B, leading to the generation of more C3 convertase. This perpetuates and amplifies complement activation enormously.

Some C3b associates with C3bBb to form C3bBbC3b, which becomes the alternative pathway C5 convertase. C5 convertase cleaves C5 to C5b and C5a. Similar to the classical pathway, C6 and then C7 bind to C5b. The C5b-7 complex inserts into the foreign membrane, followed by the binding of C8, and the assembly of C9 to form the membrane attack complex. The alternative pathway actually represents an ancient mechanism of innate immune defense. The tick-over mechanism of complement activation enables a continuous surveillance for the host, executing the first line of defense against foreign invaders.

One strategy for organisms to achieve species-specific diversities is by modification of biomolecules such as polysaccharides, glycolipids, and glycoproteins with different complexities of sugars. Typically, carbohydrate moieties on glycoproteins among vertebrates consist of complex sugars with secondary modifications (biantennary type) and ending with sialic acids. By contrast, the carbohydrate moieties in prokaryotes consist of simpler polymers of saccharides such as mannose. Pattern recognition of biomolecules is a universal theme of innate immunity.

Another ancient pathway of complement activation is initiated by the binding of pattern recognition molecules, mannan-binding lectin (MBL) or ficolins, to bacterial membranes that express arrays of simple carbohydrates such as mannose and N-acetylglucosamine (Jensenius 2005). Such binding triggers the assembly of MBL/MASP2 and ficolin/MASP2, or MBL/MASP1 and ficolin/MASP1 complex (Pathway 2, Fig. 1). MASP2 and MASP1 are mannose-associated serine proteinases. MASP2 associated with MBL or ficolin activates both C4 and C2; MASP1 activates C2 but not C4. As a result, C4b2a, a C3 convertase identical to that produced by the classical activation pathway, is generated. The downstream activation of C3 and C5 leads to the production of C3a and C5a, the formation of C5b-7 or C5b-8 sub-lytic complexes, and to the polymerization of C9 to form MAC on bacterial membranes. The ways to generate C3 convertase, C5 convertase, and MAC by the lectin pathway are identical to those of the classical pathway.

Thus, all three complement activation pathways pass through the focal point on the activation of C3 to C3a and C3b, and then C5a and C5b, leading to the assembly of sub-lytic or lytic complexes on target membranes, and production of two powerful anaphylatoxins, C3a and C5a (Fig. 1). All three pathways employ the same positive feedback mechanism that engages the generation of alternative pathway convertase C3bBb, irrespective of the original initiator, to boost up the activation process. Through interaction between the anaphylatoxins and their receptors, C3aR, C5aR (CD88), and/or C5L2, on myeloid cells including neutrophils, macrophages, and dendritic cells, the complement system cross-talks with other players of innate immunity to potentiate immune effector functions synergistically.

Complement Regulation

The complement system was achieved after millions of years of evolution. It progresses efficiently on microbial surfaces, but not on self-cells or host cells. To avoid activation that may deplete complement proteins in the circulation, and to protect from by-stander effects that may cause tissue injuries, there are regulators for every step of complement activation.

At the initiation of classical or lectin pathways, regulation is achieved by C1-inhibitor (C1-INH), a serine proteinase inhibitor that mimics the substrate for C1s in the C1 complex, or for MASP2 or MASP1 in the MBL/ficolin complex. C1-INH is cleaved by these enzymes and then becomes covalently bound to them, leading to the dissociation of the enzymatic subunit from the recognition subunit. In the absence of C1-INH, as observed among patients with hereditary angioedema, unchecked activation occurs, leading to depletions of complement C4 and C2, plus disruption of the coagulation and kinin systems. Additional regulators of complement initiation include sMAP and MAP-1 that compete with MASP2 and MASP1, respectively, for binding to MBL or ficolin, and CRIT that binds to the N-terminus of complement C2, thereby blocking the activation of C2. This prevents the formation of the classical/lectin pathway C3 convertase.

Regarding the assembly of the C3 convertases and the propagation of complement activation, there are two key mechanisms of regulation. The first is to compete with and dissociate the enzymatic subunit from the bimolecular convertases. Fluid phase protein C4b-binding protein (C4bp) dissociates the classical pathway convertase C4b2a. Complement factor H dissociates the alternative pathway convertase, C3bBb. Notably, the activity of C4bp and CFH involves recognition of host glycoproteins with glycosaminoglycans and sialic acids. Membrane-bound complement receptor CR1 and decay-accelerating factor DAF promote the disassembly of both classical pathway and alternative pathway C3 convertases on host cells.

The second complement regulation mechanism is factor I–mediated proteolytic cleavages of C4b and C3b. This factor-I digestion requires a cofactor for its activity, which is C4bp for C4b, complement factor H (CFH) for C3b, and CR1 or membrane cofactor protein (MCP) for C4b and C3b. The cleavage of C4b releases the soluble C4c, with the C4d fragment remaining covalently attached to the membrane. The factor I–mediated cleavage of C3b with CFH or MCP as cofactors leads to formation of iC3b. The cleavages of C3b by factor I with CR1 as a cofactor are more extensive, resulting in the formation of iC3b first, then soluble C3c plus bound C3dg. The latter may be further cleaved to C3d.

The anaphylatoxins generated by C3 and C5 convertases are regulated by carboxylpeptidase N (CPN). CPN removes the active site arginine residue at the C-terminus that inactivates anaphylatoxic activities of these peptides.

On the regulation of the sub-lytic and lytic complex, complement factor H–related protein-1 (CFHR1) binds to C5 and blocks its activation by C5 convertase. CRIg, complement receptor with immunoglobulin domains, regulates C5 convertase activity. The S-protein (vitronectin) blocks the insertion of C5b-7 to host membrane. Clusterin binds to C5b-7, C5b-8 and C5b-9 and

blocks the assembly of poly-C9. Protectin (CD59) on host membrane inhibits the assembly of polymeric C9 to form the lytic complex MAC by binding to C8 and C9.

Complement Receptors

There are at least 11 receptors for complement proteins. These receptors play important roles in mediating the physiologic functions of complement. Together with regulatory proteins, complement receptors facilitate the fine-tuning of complement activation with differential reactions and end-points toward self and non-self to achieve homeostasis. The binding of processed or modified complement ligands to their receptors promotes phagocytosis (opsonization), enables the trafficking and removal of apoptotic materials and immune complexes, potentiates inflammatory responses, and enhances adaptive immune response. Notably, the interactions of complement ligands and their receptors on different cell types may elicit different reactions.

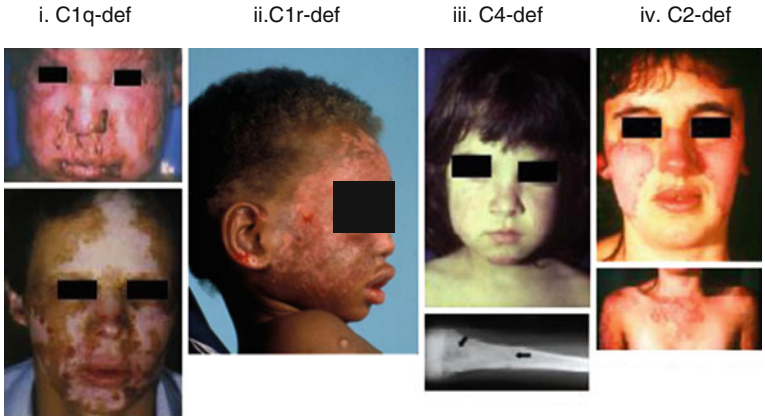
Based on the nature of ligands, complement receptors can be divided into three groups: (i) receptors for C1q and MBL, (ii) receptors for C4b and C3b and their inactivation products, and (iii) receptors for anaphylatoxins C3a and C5a.

There are three receptors that directly or indirectly bind C1q and possibly MBL. The receptor for the globular head of C1q, gC1qR appears to modulate the secretion of cytokines including IL-12 and possibly IFN-α. The other two receptors, cC1qR or calreticulin, and C1qRp or CD93, mediate phagocytosis in association with other transmembrane mediator proteins. Apoptotic cells with bound C1q are opsonized for disposal through phagocytosis by macrophages expressing C1q receptors.

There are five known receptors for C4b, C3b, and/or processed products of C3b. Complement receptor type 1 (CR1 or CD35) is a high affinity receptor for C4b, C3b, and iC3b. It is expressed on all bone marrow–derived cells including human red blood cells, lymphocytes, myeloid cells, and follicular dendritic cells of the peripheral lymphoid organs. CR1 on human erythrocytes picks up immune complexes coated with C4b, C3b, or iC3b for clearance in the liver and spleen. CR1 on neutrophils and macrophages promotes phagocytosis of antigens with C4b, C3b, and iC3b. As mentioned previously, CR1 is a cofactor for factor I–mediated proteolysis of C4b to C4c and C4d, and C3b to iC3b, C3dg and C3d. C3dg and C3d are ligands for complement receptor type 2 (CR2 or CD21). CR2 is a subunit of the tri-molecular complex, CD19-CD21-CD81, which is the co-receptor for B cell activation. The binding of either C3d- or C3dg-coated antigens or immune complexes to CR2 on B cells lowers the threshold of B cell activation and differentiation. CR2 on follicular dendritic cells serves to trap and retain antigens or immune complexes coated with C3d or C3dg in the peripheral lymphoid organs, a process important for immune memory and secondary immune response of humoral immunity (Carroll and Isenman 2012).

Complement receptors CR3 (ITGAM- or CD11b-CD18) and CR4 (ITGAX- or CD11c-CD18) belong to the β_2 integrin family. These receptors are both heterodimers, each with a distinct α-chain and a common β-chain (CD18). Besides iC3b, ligands for these receptors include intercellular adhesion molecule 1 (ICAM-1; CD54), fibrinogen and lipopolysaccharides (LPS). Both receptors are expressed on myeloid cells. They are stored in vesicles and mobilized to cytoplasmic membranes when myeloid cells are activated. In addition to promoting adhesions of myeloid cells to vascular endothelia at sites of inflammation, CR3 and CR4 are engaged in phagocytosis of iC3b-opsonized antigens. CR3 also interacts with toll-like receptors (TLR2, 4 and 9) in fine-tuning cytotoxic functions and end-points of innate immunity. Moreover, the cytoplasmic domain of CR3 provides signaling functions for glycosylphosphatidylinositol-anchored membrane proteins including Fc-γ receptor IIIb (FCGR3B or CD16b).

CRIg, the complement receptor with immunoglobulin-fold, is a receptor expressed in resident macrophages such as the Kupffer cells in the liver. This receptor binds to and mediates phagocytosis



Complement in Rheumatic Diseases, Fig. 2 Homozygous complement deficiency in human lupus. Severe cutaneous lesions are common clinical presentations in lupus patients with a complete deficiency of a classical pathway component protein. (i) A homozygous C1q-deficient male child with lupus erythematosus (LE) symptoms and cutaneous infection (*upper panel*), and with discoid LE and scarring lesions on face when he was 22 years old (*lower panel*). (ii) A homozygous C1r-deficiency male child with discoid lupus at 16 months old. This patient experienced generalized seizure, developed scissoring gait with toe walking, spasticity, and weakness of the legs. At 18 years old, he was diagnosed with class IV lupus nephritis and progressed to end-stage renal disease. (iii) A complete C4-deficient girl at 3 years old with butterfly rash and cheilitis (*upper panel*), and osteomyelitis of the femur at 10 years old (*lower panel*). This patient died at age 12 because of pulmonary infection and cardiovascular failure. (iv) A homozygous C2-deficient young woman with acute cutaneous LE. The *upper panel* shows the butterfly rash, the *lower panel* shows photosensitive lesions on sun-exposed area (Source of pictures: Yu CY et al. A Companion to Rheumatology-Systemic Lupus Erythematosus, 1st edition, Mosby Elsevier, Philadelphia, p185, 2007; Wu YL et al., Lupus 20:1126–1134, 2011)

of C3b or iC3b-opsonized particles. Therefore, CRIg is important in immunoclearance.

There are three known receptors for anaphylatoxins C3a and C5a, which are C3aR, C5aR (CD88) and C5L2 (GPR77). These receptors are characterized by the presence of seven transmembrane domains. C3aR and C5aR are G-protein coupled receptor proteins and engaged in proinflammatory signaling and effector functions upon ligations to C3a and C5a, respectively. C5L2, which binds to both C5a and C5adesArg, is independent of G-protein coupling. C5L2 is probably a decoy receptor of C5aR (Klos et al. 2013).

Genetic and Acquired Deficiencies of Classical Pathway Complement Proteins in Systemic Lupus Erythematosus (SLE)

The intricate roles of complement in rheumatic diseases are reflected by three series of observations (Atkinson and Yu 2011; Sturfelt and Truedsson 2012; Yu et al. 2007). First, homozygous genetic deficiency of any complement components specific for the classical pathway of activation (C1q, r, s, and C4) is a likely, albeit rare, cause of human SLE (Fig. 2). Second, partial or acquired deficiencies of C1q, C4 or C2 are present in numerous SLE patients. Such partial or acquired deficiencies can occur because of the presence of (i) a structural genetic variation including gene copy number variation or a polymorphism that affects the protein expression level or its functional activity, (ii) autoantibodies against C1q, (iii) a deficiency of complement C1 inhibitor leading to chronic activation and depletion of C4 and C2, or (iv) a side effect of drugs, such as from hydralazine derivatives, which inactivates the complement C4 thioester bond. Third, low levels of plasma complement C4 and/or C3 are frequently observed among SLE patients, particularly at disease diagnosis or during disease flares.

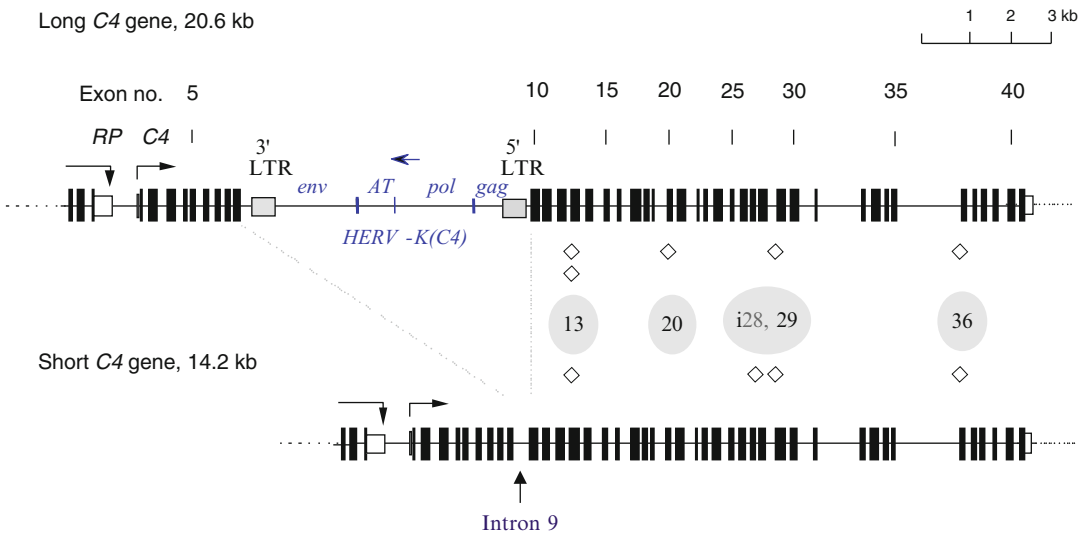
In a considerable proportion of SLE patients, serum C4 and C3 protein levels are persistently low, regardless of treatment.

Genetic Deficiency of Total C4 in Human SLE. A complete or homozygous genetic deficiency of complement C4A and C4B has been reported in 28 individuals (Atkinson and Yu 2011). Of these C4-deficient subjects, 22 (78.6 %) were diagnosed with SLE or a lupus-like disease, while 4 others had renal disease including glomerulonephritis. These C4-deficient subjects came from 19 families with different racial backgrounds, and were characterized by 16 different haplotypes of the major histocompatibility complex (MHC, also known as the HLA). Of the C4-deficient SLE patients, the female to male ratio was 1:1. Early disease onset, severe photosensitive skin rash, the presence of autoantibodies against ribonuclear protein Ro/SSA, and high titers of antinuclear antibodies (ANA) were common clinical features. Many C4-deficient patients also had severe proliferative glomerulonephritis. Almost all complete C4 deficiency patients are homozygous with identical HLA class I and class II markers from both copies of chromosome 6, possibly because of consanguineous marriages. The molecular basis of complete C4 deficiency had been determined in 15 cases. Details of the defects are shown in Fig. 3.

Copy Number Variation (CNV) of C4 Genes and Deficiency of C4A in SLE. An apparent phenotypic deficiency of complement C4A or C4B has been observed in a large proportion of SLE patients. However, the underlying causes for such phenotypes had been controversial until recently, largely because of the unawareness of common copy number variations (CNVs) and associated polymorphisms for *C4A* and *C4B* genes. Located in the central region of the HLA, among different individuals, there can be one, two, three, or four copies of *C4* genes on each chromosome 6. As a result, there is a continuous variation in the gene copy number (GCN) of C4 genes from 2 to 8 in a diploid genome among different individuals (Fig. 4). The multiplication of a *C4* gene is always concurrent with three neighboring genes. This phenomenon is dubbed RCCX modular duplications. Each RCCX module consists of a gene fragment corresponding to the 3′ end RP (also known as *STK19*), an intact *C4* gene, an intact steroid *CYP21* gene, and another gene fragment corresponding to the 3′ end of tenascin *TNX*. Each *C4* gene in the RCCX module either codes for an acidic C4A protein or a basic C4B protein. Each *C4* gene can either be a long gene of 20.6 kb or a short gene of 14.2 kb, in size. The long gene is due to the integration of an ancient retrovirus HERV-K(C4) of 6.36 kb into the ninth intron of the *C4* gene (Fig. 3).

A comprehensive study has been performed in the USA to rigorously investigate the complement *C4* genetic diversities in SLE of European decent and race-matched controls. The primary study population included 1,241 European-Americans with 233 SLE patients and 356 first-degree relatives, plus 517 unrelated healthy controls (Yang et al. 2007). Long-range mapping technique using pulsed field gel electrophoresis was employed to resolve large DNA fragments containing all RCCX modules or the number of *C4* genes present in each haplotype. Regular genomic restriction fragment length polymorphisms were performed to decipher the fine details of RCCX constituent genes. The C4 protein phenotypes were elucidated by immunofixation of EDTA-plasma, immunoblotting analyses using monoclonal antibodies to determine the Rodgers and Chido blood group antigens associated with C4A or C4B proteins, and radial immunodiffusion to determine the plasma C4 protein concentrations (Chung et al. 2005). In this particular study population, the gene copy numbers of total *C4* varied from 2 to 6, *C4A* from 0 to 5, and *C4B* from 0 to 4. Their corresponding median copy numbers were 4, 2, and 2, respectively. In comparison to healthy controls, SLE patients had their total *C4* and *C4A*, but not *C4B*, gene copy number groups shifted to the lower copy number side (Fig. 4).

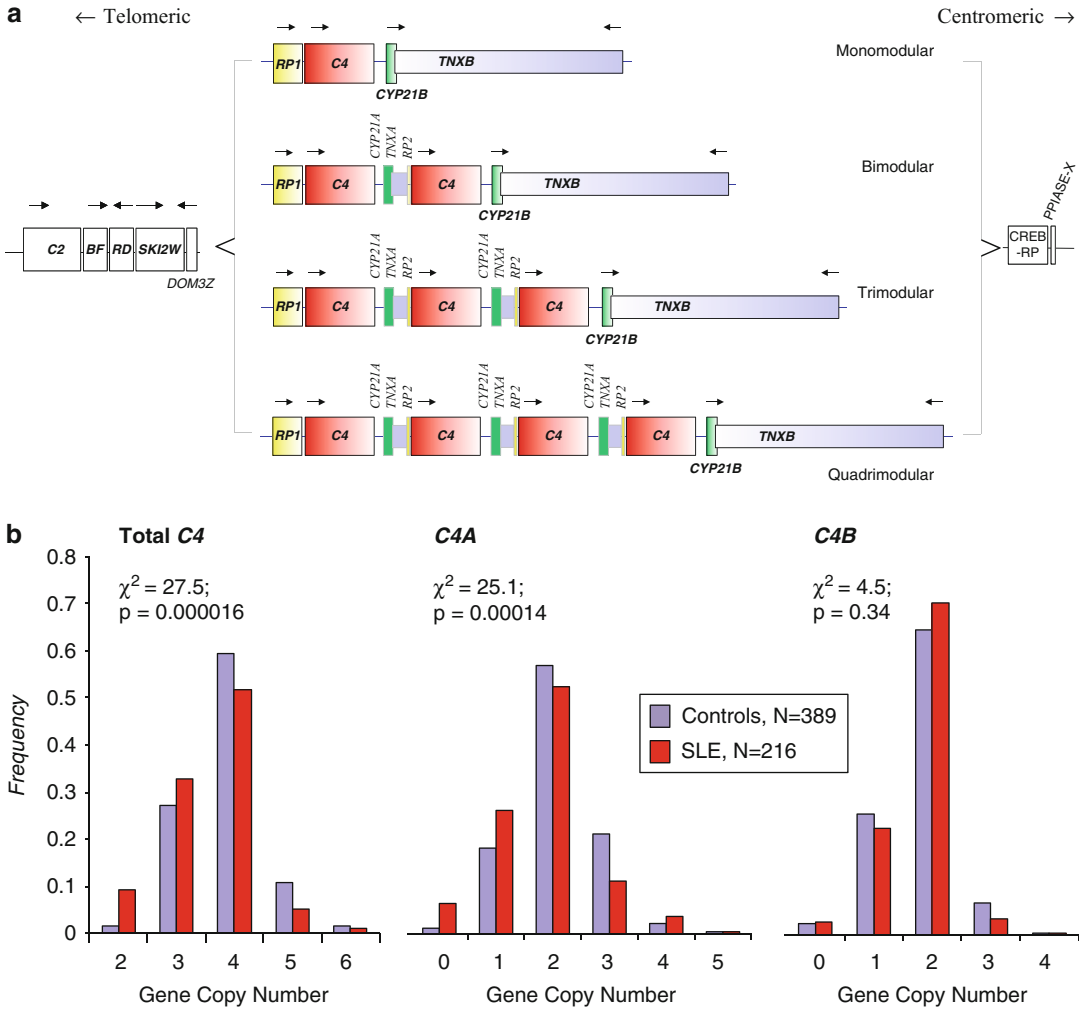
Among female SLE patients, 9.3 % had only two copies of *C4* genes, and 6.5 % had a homozygous deficiency of *C4A*, compared to 1.5 % and 1.3 %, respectively, in healthy controls. Among the subjects with only two copies of *C4* genes,



Exon 13 (9)		
Codon 497, 2-bp deletion, ***C4AQ0***	RCCX: mono-L	HLA *A24 Cw7 B38 DR13*
Codon 522, 1-bp deletion, *C4AQ0* -***C4BQ0***	RCCX: LS	HLA *A2 B12 DR6*
Codon 540, C→T nonsense mutation, ***C4AQ0***	RCCX: mono-L,	HLA *A2 B17 DR7*
Exon 20 (1)		
Codon 811, 1-bp deletion, ***C4AQ0***	RCCX: mono-L	HLA *A30 B18 DR3*
Intron 28, (4)		
Donor site, G→A mutation, ***C4BQ0-C4BQ0***	RCCX: SS	HLA *A30 B18 DR7*
Exon 29 (5)		
Codon 1213, 2-bp insertion, ***C4AQ0***	RCCX: mono-L	HLA *A2 Cw3 B40 DR6*
C4AQO-*C4BQ0*	RCCX: LS	HLA *A2 B12 DR6*
C4AQ0-C4BQ0	RCCX: LS	HLA *A2 Cw7 B39 DR15*
Exon 36 (1)		
Codon 1537, 4-bp insertion, ***C4AQ0-C4BQ0***	RCCX: LS	HLA *A1 B17 DR13*

Complement in Rheumatic Diseases, Fig. 3 Molecular basis of complete C4 deficiency. A total of 28 subjects are known to have complete C4 deficiency. Among them, the molecular bases for the non-expression of C4 proteins in 15 subjects have been determined. These subjects were from eight different HLA haplotypes. Molecular defects in the mutant *C4* genes include nonsense mutations caused by point mutations, mini-deletions and mini-insertions in the coding regions, and point mutations at intron/exon splice junctions. *Filled black boxes* are the 41 exons in each *C4* gene. HERV-K (C4) is an endogenous retrovirus inserted into the intron 9 of long *C4* genes (Source: Wu YL et al., Genes and Immunity 10:433–445, 2009)

a great majority of them had no *C4A* genes; a small proportion of subjects had one copy of *C4A* and one copy of *C4B*, or with two copies of *C4A* and no *C4B*. The odds for SLE disease risk (or odds ratio, OR) for a subject with only two copies of *C4* genes in a diploid genome are 6.5 times greater than those with three or more copies of *C4* genes. Among the subjects with no *C4A* genes, the odds for SLE disease risk are 5.7 times greater than those with one or more copies of *C4A* genes. In other words, *very* low gene copy number (GCN) of total *C4* (GCN = 2), or



Complement in Rheumatic Diseases, Fig. 4 Copy number variation (CNV) of complement *C4* genes in SLE and controls. (**a**) RCCX modular variations. The *C4* gene is located in the class III region of the HLA on chromosome 6p21.3. On each chromosome 6, there can be one to four copies of *C4* gene. The duplication of a *C4* gene is concurrent with its three neighboring genes *RP* (*STK19*), *CYP21* and *TNX*. Thus, the phenomenon is known as RCCX duplication. Each *C4* gene codes either for an acidic C4A protein or a basic C4B protein. (**b**) Comparisons of total *C4*, *C4A*, and *C4B* gene copy numbers in SLE and controls. SLE patients show significantly lower copy numbers of total *C4* and *C4A* (Modified from Atkinson JP and Yu CY, In Systemic Lupus Erythematosus, 6th Edition, Lahita R, Tsokos G, Buyon J and Koike T eds, Academic Press, Amsterdam, pp21-45, 2011)

a homozygous deficiency of *C4A* (GCN = 0), is a large effect size (strong) genetic risk factor for human SLE.

Parallel increases in frequencies of heterozygous C4A deficiency (GCN = 1), and moderately low copy number of total C4 (GCN = 3), were observed in the SLE population. However, their effect size or impact on disease risk is substantially lower. Specifically, the frequency of subjects with a single copy of *C4A* was 26.4 % in SLE and 18.2 % in controls (OR = 1.6); the frequency of subjects with moderately low copy number of total *C4* was 32.9 % in patients and 27.0 % in controls (OR = 1.3). On the contrary, human subjects with high copy numbers of total *C4* or *C4A* genes were protected

against SLE disease risk (total *C4*, ≥5 copies: 6.0 % in patients, 12.0 % in controls; OR = 0.47; *C4A*, ≥3 copies: 15.3 % in patients, 23.8 % in controls; OR = 0.57) (Yang et al. 2007). In an independent study of SLE in Han-Chinese, significantly lower gene copy number of C4A and total C4 genes were observed in the SLE cases than matched controls (Lv et al. 2012).

Secondary or Acquired Deficiency of C4 and Susceptibility to SLE. Inherited or acquired deficiency of the complement C1 inhibitor is associated with an increased frequency of autoimmunity, especially SLE (Agnello 1986). With a deficiency of the C1 inhibitor, C1 cleaves, in an unchecked fashion, its natural substrates, C4 and C2, causing a chronic marked reduction in their blood levels. In most patients with C1 inhibitor deficiency, C4 and C2 concentrations are continuously reduced such that it simulates a C4-deficient and a C2-deficient state.

Therapeutic agents hydralazine and isoniazid may also induce SLE through inhibition of C4 function. The concentrations of hydralazine and isoniazid that inhibited the activity of C4 by 50 % were in the range of those obtained during therapy. Related to this observation was the finding of an increased frequency of C4A null alleles, which already predisposes a subject to higher risk of autoimmunity, in hydralazine-induced SLE patients (Sim et al. 1984).

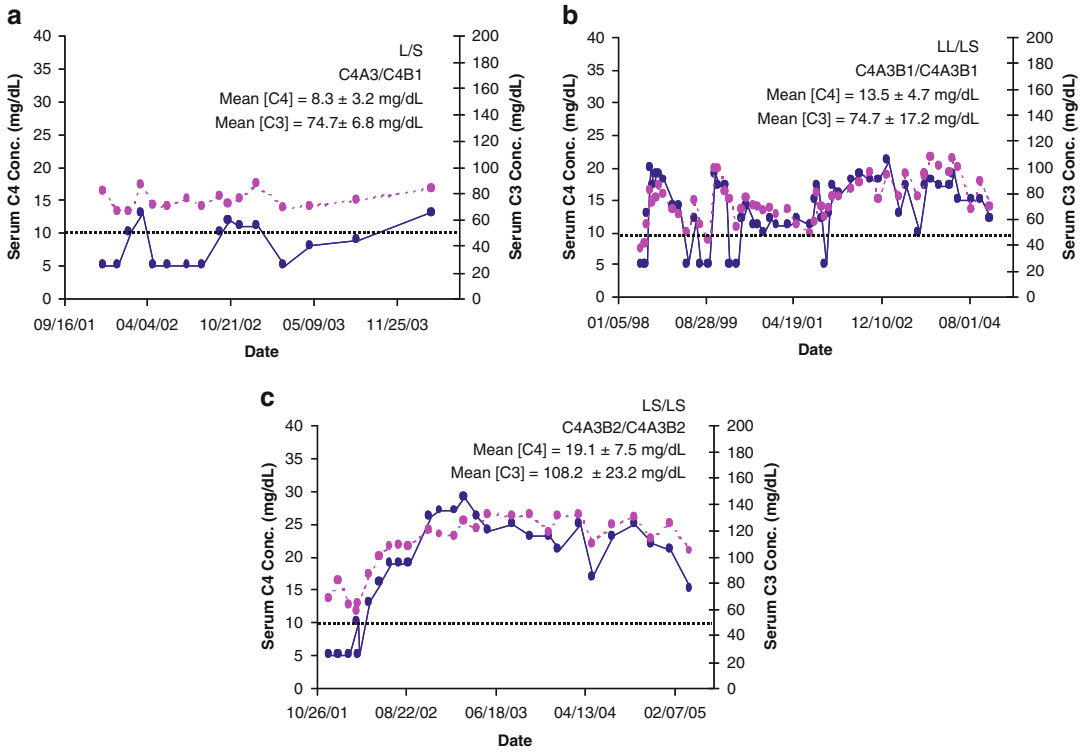
C4 Protein Concentrations in Serum and C4 Cleavage Products on Blood Cells. SLE patients are often characterized by occasionally or persistently low serum or plasma levels of complement C4 and/or C3. Through multiple logistic regression analyses, several parameters that determine serum or plasma C4 protein concentrations have been identified. The *C4* gene copy number, the copy number of short *C4* genes, and the body-mass index are positively associated with increased C4 protein concentrations. The presence of SLE is negatively associated with C4 protein levels. In some, but not all, of such patients, complement C3 and C4 levels are biomarkers for lupus disease activity – low serum levels of C3 and/or C4 correlate with a flare while normal C3/C4 levels tend to correspond with a disease remission.

Longitudinal studies of serum complement protein levels in SLE patients reveal different expression profiles (Fig. 5) (Wu et al. 2006). In one group of patients, persistently low C4 and C3 protein levels were detected, and many of these patients had a low copy number of *C4* genes. The second group of patients was marked by frequent fluctuations of C4 and C3 protein levels and SLE disease activity. The third group of patients was characterized by occasionally low C4 and C3 levels, particularly at the time of disease diagnosis, but their levels often returned to normal. Most patients in the second and third group had the median or higher copy numbers of total *C4* or *C4A*, and the C4 and C3 protein concentrations roughly parallel the SLE disease activities.

Another way to observe the consumption of C4 in SLE is by the presence of degradation product C4d attached to red blood cells, reticulocytes, and platelets (Liu et al. 2009). This chronic activation of C4 can be monitored by flow cytometry of peripheral blood samples using antibodies against C4d. It is suggested that levels of RBC-C4d reflect complement activation in the past 60 days, the levels of reticulocyte-C4d reflect during the past 2–3 days, and platelet-C4d levels reflect complement activation during the past 5 to 10 days.

Genetic Deficiency of C1q. The a, b, and c chains of human C1q are encoded by three different genes closely linked on chromosome 1p34-1p36. A total of 42 human subjects have been identified with a genetic deficiency of C1q. Among them, 39 (92.9 %) developed a syndrome related to SLE. The female to male ratio in C1q-deficient SLE was close to 1:1. The median age disease onset was 6 years old (range: 6 months to 42 years old).

C1q-deficient SLE patients were characterized by skin rash (86 %) (Fig. 2), glomerulonephritis (GN) (38 %), and central nervous system (CNS) involvement (17 %). For autoantibodies, the prevalence of ANA was only 70.6 % (24/34), anti-dsDNA was 20.8 %, and anti-ENA (extractable nuclear antigens including Sm, RNP, Ro, and/or La) was 62.5 % (15/24). At least one-third of the C1q-deficient patients were inflicted with recurrent bacterial infections



Complement in Rheumatic Diseases, Fig. 5 Typical C4 and C3 serial protein profiles in human SLE patients. Serum C4 and C3 protein levels tend to go up and down together in many SLE patients. In the first group of patients (*panel A*), levels of C4 and C3 are chronically low. In some patients, even if C3 levels rise to normal range, C4 levels remain low. This group of patients is characterized by low copy number of C4 genes. The second group of patients has frequent fluctuations of C3 and C4 (*panel B*). This group of patients has active disease, and low C3 and low C4 roughly correlate with disease activity. In the third group of patients, C4 and C3 protein levels stay in the normal range most of the time, except at the time of diagnosis and during a disease relapse. These patients tend to have relatively inactive disease. The second and third groups of patient have normal gene copy number of total *C4* but may have a heterozygous deficiency of *C4A* (Source: Wu YL et al. Adv Exp Med Biol 586:227–247, 2006)

including otitis media, meningitides, and pneumonia. Four C1q-deficient patients died of septicemia in early childhood. Some patients also developed diffuse monilia and aphthous lesions in the mouth and toenail deformity secondary to moniliasis.

There are two major causes for a hereditary C1q deficiency. The first is a nonsense mutation resulting in a failure to synthesize the C1q protein, which accounts for ~60 % of cases. Such nonsense mutations occur at codons 6 or 41 and frame-shift mutations from codon 43 that culminate to a stop codon at residue 108 of the C-chain; a stop codon for residue 150 of the B-chain; and a stop codon for residue 186 of the A-chain. The second cause of C1q deficiency is the synthesis of a low molecular weight, nonfunctional C1q, which accounts for ~40 % of the cases. Low molecular weight C1q can be caused by missense mutations that disrupt the collagen motifs in C1q and interfere with the formation of higher order structure of C1q. A normal C1q consists of 18-polypeptides intertwined to form a tulip-like structure with six globular heads and a collagen-like tail (Lu et al. 2008).

Hereditary and Acquired Hypocomplementemia of C1q. In addition to the coding mutations leading to the absence of C1q protein biosynthesis or a production of nonfunctional

C1q protein, it was observed that a silent single nucleotide polymorphism at codon 70 (GGG→GGA) of the *C1QA* gene was associated with decreased levels of C1q in patients with subacute cutaneous lupus erythematosus. Whether the SNP at codon 70 is engaged in the regulation of gene transcription has not been clarified.

Distinct from inborn factors leading to low levels of C1q production, acquired hypocomplementemia of C1q due to an increased protein consumption is frequently observed in SLE patients or other autoimmune conditions, including hypocomplementary urticarial vasculitis syndrome, cryoglobulinemia, and severe combined immunodeficiency syndromes. The major underlying cause is the presence of autoantibodies against C1q. C1q autoantibodies are present in roughly one-third of SLE patients, particularly among those with renal diseases and high disease activities.

Genetic Deficiency of C1r or C1s. Deficiencies in subcomponents of the C1 complex, C1r and/or C1s, were among the earliest reported linking complement deficiency with human SLE. The genes for human *C1s* and *C1r* are located on chromosome 12p11.31. These two genes are configured in a tail-to-tail orientation with their 3′ ends separated by 9.5 kb. The human *C1s* gene spans 10.5 kb with 12 exons coding for a precursor protein of 688 amino acids, which includes a leader peptide of 15 amino acids. The human *C1r* gene probably also consists of 12 exons.

A total of 20 cases of C1r and/or C1s deficiencies (12 cases of C1r deficiency from 8 families; 8 cases of C1s deficiency from 5 families) have been documented so far. Among these subjects, all but three were inflicted with recurrent bacterial, viral, or fungal infections. Thirteen C1r-/C1s-deficient subjects developed SLE or a lupus-like disease (65 %). The female to male ratio among C1r-/C1s-deficient subjects with SLE was 1.5 to 1. It is noteworthy that several "non-SLE" C1r- or C1s-deficient subjects were identified because of severe infections but died at young age and were therefore not available for follow-up examinations of autoimmune disease status.

Most C1r-/C1s-deficient patients were characterized with severe cutaneous lesions. Eight patients had renal disease due to lupus nephritis (40 %) (Fig. 2). Relatively low prevalence of ANA (59.4 %) was found. Among the C1r-deficient patients, there was consistent reduction in the serum protein levels of C1s to ~30 % of its normal levels, but highly *elevated* protein levels of complement C4, C2, and C1 inhibitor (200–400 % of the normal ranges), and unaffected expression level of C1q. A similar phenomenon was observable among C1s deficient patients: greatly reduced serum levels of C1r, elevated levels of C4, and steady levels of C1q. Such presentations underscore the interdependence of C1r and C1s in sustaining a stable tetrameric structure that would otherwise be susceptible to high turnover rate. A deficiency of C1r or C1s incapacitates the formation of the C1 complex and the ensuing activation of the classical activation pathway. An impairment of C1r or C1s therefore greatly reduces the consumption of C4 and C2 and the engagement of C1 inhibitor in regulating the formation of C1 complex, resulting in unusually high levels of these proteins in the circulation.

The molecular defects leading to C1r or C1s deficiency have been determined in four patients. Among them are a homozygous C to T mutation in exon 10 of the *C1r* resulting in a R380X nonsense mutation, a 4-bp deletion in exon 10 of *C1s* that created frame-shift mutations and formation of a premature stop codon, and two different nonsense mutations at codon 534 or codon 608 in exon 12 of *C1s*.

Complement C2 Deficiency and Susceptibility to SLE. Complement C2 deficiency occurs with an estimated prevalence of 1/20,000 in individuals of northern European descent. Complete C2 deficiency accounts for <1 % of SLE patients. C2-deficienct SLE patients tend to have early childhood onset but a milder disease process, with prominent photosensitive dermatologic manifestations, speckled autoantibodies that are common for the Ro (SSA) antigen, and a familial history of SLE (Fig. 2). Anti-DNA antibody tests were mostly negative. Severe kidney diseases were relatively rare among C2-deficient patients.

The association of C2 deficiency with human SLE is considerably different from that observed for C4 or C1 component protein deficiencies. First, the prevalence of SLE among C2-deficienct subjects is only between 10 % and 30 %. In other words, the effect size of C2 deficiency on SLE is substantially lower than that of C4 or C1 deficiencies. Second, the female predominance of SLE in C2-deficienct approaches that observed in the general SLE cases. Third, the severity of disease is not distinct from that of the general lupus population.

There are two mechanisms leading to complement C2 deficiency. Type 1 C2 deficiency is caused by nonsense mutations leading to the absence of C2 protein biosynthesis. The predominant form of Type 1 deficiency is present in the HLA *A10 (A25), B18, DRB1*15 (DR2), BFS, C2Q0, C4A4, C4B2*, haplotype. The defect is caused by a 28-bp deletion that removes 9-bp from the 3′ end of exon 6 and 19-bp from the 5′ end of intron 6, leading to a skipping of exon 6 in the *C2* transcript and generation of a premature stop codon. The second form of Type 1 C2 deficiency is due to a 2-bp deletion in exon 2 that leads to a nonsense mutation, as observed in HLA *A3, B35, DR4, BFF, C2Q0, C4A3A2*.

About 10 % of C2 deficiency belongs to the Type 2 deficiency, in which the C2 protein is synthesized but not secreted. The molecular defects were found to be missense mutations leading to the following changes: C111Y, S189F, and G444R. It is unclear how these missense mutations block the secretion of C2.

Genetic Deficiency of Classical Pathway Complement Proteins in Human and Murine Lupus

The prevalence of SLE among subjects with a genetic deficiency of complement C4 or component proteins (C1q, r, s) of the C1 complex (65–93 %) is higher than that observed among monozygotic twins (24–58 %) (Tsao and Wu 2007). The common features among complement-deficient SLE are the early age of disease onset, the roughly equal prevalence in males and females, the presence of anti-Ro/SSA, and severe cutaneous disease (Lipsker and Hauptmann 2010). These data suggest a causal role of complement deficiency in SLE. Although linked in the same activation pathway that is triggered by the binding of antibodies to antigens, these classical pathway complement proteins are *independent* in the protection against SLE disease susceptibility, as a deficiency in any of these four complement proteins is strongly linked to lupus disease susceptibility. The molecular defects leading to a genetic deficiency of C1q, C1r, C1s, or C4 are mostly *private* mutations detectable in probands and their close relative (s), but rarely in the general SLE population. Such a phenomenon is in keeping with the theory of "rare genetic variants with very large effect size in common diseases" (McClellan and King 2010).

The overwhelming roles of classical pathway complement protein deficiency in SLE pathogenesis have been tested in mouse models with genetic ablations of complement genes (Holers 2000; Pickering et al. 2001). In permissive genetic backgrounds, C1q-deficient or C4-deficient mice showed enhanced lupus phenotypes. Lupus disease onset was generally earlier. Antinuclear antibody titers were higher. There were also higher frequencies of DNA antibodies, and more pronounced glomerulonephritis. It is of interest to note that mice deficient in factor B, C2, or C3 did not develop or show enhanced spontaneous SLE phenotypes under any genetic background tested. It is also noteworthy that a spontaneous lupus disease phenotype in C4-knockout or C1q-knockout mice was strain dependent. Unlike the great degree of association between C4 deficiency or C1q deficiency and human SLE from multiple independent families and different racial backgrounds, a pronounced lupus phenotype was not observed in mice of most genetic backgrounds with C4 deficiency or C1q deficiency, except in those with lupus-prone backgrounds.

Two major hypotheses have been put forward to explain the engagement of complement in autoimmunity and tolerance. The first, mostly known as the waste disposal hypothesis

(Korb and Ahearn 1997; Taylor et al. 2000), is based on the roles of activated complement including C1q, C4b, and C3b in opsonizing immune complexes, foreign particles and apoptotic, necrotic, netotic, or damaged self-materials for phagocytosis and clearance from the circulation, and on solubilizing aggregates of immune complexes and reducing cryoglobulin activity. Therefore, the chance of generating autoantibodies or autoreactive T cells against self-antigens is minimized. While physiological, this hypothesis does not explain some most fundamental phenomena connected to human autoimmune diseases. Processed products of C3b including iC3b, C3dg, and C3d are highly relevant in opsonizing immune complexes and foreign particles for phagocytosis through binding to complement receptors CR1, CR3, and possibly CR4, and for B cell development through binding to CR2. However, it is C4 deficiency, and not C3 deficiency, that is strongly linked to the predisposition of SLE, both in humans and mice. While it is established that C1q binds to late apoptotic blebs and facilitates their removal through phagocytosis by macrophage or dendritic cells, MBL probably plays a similar role, but MBL deficiency does not elicit strong autoimmune defects like C1q deficiency does in humans. In addition, why a deficiency of C1r or C1s (but not MASP2 or MASP1) strongly predisposes a subject to SLE pathogenesis in the presence of normal C1q and very high C4 levels is of question.

The second hypothesis entails the observations of complement in B cell activation in animal model studies (Carroll and Isenman 2012). It has been observed that complement-coated immune complexes or antigens are trapped by follicular dendritic cells in the spleen or peripheral lymphoid system. Through germinal center reactions, antigens are presented to helper T cells, which lower the thresholds of B cell activation and promotes class-switching from low specificity and low affinity IgM to high specificity and high affinity IgG. Some complement-coated antigens are retained for long periods of time and are sampled continuously by dendritic cells in the peripheral lymphoid system. This helps explain the roles of complement in facilitating the secondary immune response and achieving immune memory. These reactions mostly engage the binding of C3b and/or C4b to CR1, C3b or iC3b to CR3 on dendritic cells and phagocytes, and C3d- or C3dg-coated antigens to CR2 on B cells. CR2 is an important co-receptor for B cell activation. It is possible that a complement-deficient or -depleted host would have prolonged or chronic antigenemia, as well as accumulation of modified or damaged self-antigens in the circulation. One probable outcome would be ectopic or impaired follicular localizations of autoantigens, leading to aberrant immune response against self-antigens. Again, this hypothesis relies on the prominent roles of C3b and its processed products on B and T cell activations. Similar to the waste disposal hypothesis, this complement in B cell activation hypothesis is unable to provide a satisfactory explanation on the differential roles of C4 deficiency and not C3 deficiency in the pathogenesis of systemic autoimmune diseases.

The classical pathway of complement activation has evolved to be a downstream effector for the adaptive humoral immune response (Fig. 1). It would be natural, and actually essential, for this effector arm to have developed a concurrent feedback mechanism to prevent or downregulate the generation of antibodies against self-antigens to achieve tolerance. Irrespective of the sequence or the hierarchy in the activation pathway, C1q, C1r, C1s, and C4 are all needed to prevent the occurrence of systemic autoimmunity against ubiquitous nuclear and cytoplasmic antigens. The emerging concept is that activated complement proteins of the classical pathway are engaged (directly or indirectly) in the regulation or the homeostasis of adaptive immunity, including the discrimination between self- and non-self-antigens.

There are multiple triggers and independent routes leading to the initiation of autoimmune rheumatic diseases. Among human subjects with an established autoimmune disease that engages autoantibodies, or with an inflammatory disease, complement activation and consumption is of concern. The generation of anaphylatoxins C3a and C5a creates an inflammatory state that

attracts and activates neutrophils and other myeloid cells to the site of complement activation. The formation of the membrane attack complex likely causes tissue damage and aggravates disease severity. Among SLE patients with an active disease or with lupus nephritis, presence of low levels of serum C4 and C3, and high levels of cell-bound C4d, is a general phenomenon. The harmful effects of complement activation are even more remarkable in rheumatoid arthritis.

Complement in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic, inflammatory, rheumatic disease affecting 0.1–1 % of the human population worldwide. RA is characterized by an inflammatory process that targets the synovium of primarily peripheral joints, leading to irreversible joint destruction and disability, plus systemic or extra-articular complications (McInnes and Schett 2011; Okroj et al. 2007). In the RA patients, there are marked infiltrations of the synovial linings with T and B cells, macrophages, and granulocytes, leading to swollen joints, destruction of cartilages, bone resorption, revascularization, and deformity.

Autoantibodies play a major role in the more severe forms of RA. The autoantibodies that are best characterized in RA are: (i) autoantibodies against citrullinated proteins (ACPA) that are both specific and sensitive, and (ii) autoantibodies against the Fc-fragment of immunoglobulins (rheumatoid factor, RF) that are sensitive but not specific. RA patients whose blood tests classified as seropositive typically express both RF and ACPA. The strongest genetic risk factor for RA is present in HLA, particularly polymorphic variants of *DRB1* genes in the class II region. More refined studies have showed that about 85 % of the seropositive RA patients have the shared epitope with amino acid sequence [(R/Q)(K/R)RAA] at position 70–74 of the HLA DR-β chain, which are mainly encoded by specific subtypes of *DRB1*04*. Seropositivity and the presence of shared epitope appear to confer increased risk of RA. In a recent study on HLA class III genes in RA, it was found that the frequency of homozygous and heterozygous deficiency of complement C4B was significantly increased in the seropositive RA patients, suggesting that C4B deficiency interacts with shared epitopes in the development of seropositive RA (Rigby et al. 2012).

Regardless of the genetic and environmental risk factors that initiate the onset of RA, it is well documented that complement proteins play important roles in the clinical phase of the disease. Complement component proteins and their proteolytic cleavage products are present in synovial fluids. Synovial fluids from RA patients contain microparticles that are likely released by apoptotic or necrotic cells. These microparticles are coated with C1q and cleavage products C3d and C4d, suggesting chronic complement activation that is likely initiated by the formation of immune complexes between RA-associated autoantibodies and autoantigens, activation of the classical pathway, and ensuing amplifications by the alternative pathway. Depressed levels of component proteins, elevated levels of complement cleavage products, and membrane attack complex C5b-9 are noted in synovial fluids from RA patients with active disease. Because of the under-expression of the terminal pathway regulatory protein CD59, the synovial membrane is particularly vulnerable to membrane attack complex–mediated damage (Okroj et al. 2007). Neutrophils and fibroblasts in synovial fluids have high expression levels of complement receptors on their membrane. Sublytic levels of C5b-9 activate synovial fibroblasts to secrete collagenases and metalloproteinases, which degrade collagens. Elevated levels of anaphylatoxins C3a and C5a in synovial fluid attract the migration of neutrophils to the inflamed joints, and activate neutrophils, macrophages, mast cells, and fibroblasts through binding to C5aR or C3aR. The secretion of inflammatory cytokines, degranulation and release of reactive oxygen species, vasoactive peptides, histamines, chemokines, proteolytic enzymes including metalloproteases collectively

contribute to joint erosion, swollen joints, and leakage of plasma proteins from micro-vessels (Okroj et al. 2007; Ricklin et al. 2010; Sturfelt and Truedsson 2012).

The relevance of complement activation in RA has been validated in animal models. Rats depleted of complement C3 through cobra venom factor were resistant to the development of collagen-induced arthritis (CIA). A variety of genetically engineered mouse strains with complement C5 deficiency or C5a-receptor deficiency were resistant to CIA, while complement C3 deficiency or factor B deficiency were either RA resistant or had a milder disease. Intriguingly, deficiencies in C1q, C4, or C6 had no effects on the progression of induced arthritis in rodent models. This phenomenon emphasizes the significance of complement activation by the alternative pathway or proteinases for the generation of anaphylatoxins C5a and C3a in the *clinical* phase of RA in animal models. Thus, applications of complement inhibitors at the level of C5 and/or C3 activations, and antagonists or blockage of C5aR and C3aR appear to be logical therapeutic approaches to treat or attenuate the disease. Indeed, there are ongoing therapeutic studies for RA in animal models using monoclonal antibodies against C5 to block C5 activation, compstatin to block C3 activation, or soluble complement receptor analog (sCR1) with complement controlling protein (CCP) domains to competitively inhibit the formation of C3 or C5 convertases. Many of these complement inhibitors appear to reduce the disease severity and halt the progression of an established disease in animal models, opening the door for potential clinical trials.

Conclusion

The complement system is activated through three distinct pathways. The alternative pathway forms the first line of defense against infections. It is initiated through a tick-over mechanism that activates complement C3 by continuous hydrolysis in the circulation. The lectin pathway engages pattern recognition by MBL and ficolins of glycosylated microbial antigens with simple sugar molecules. The classical pathway is activated by antibodies binding to antigens in adaptive humoral immune response. All three activation pathways converge on the activations of C3 and C5; the end-point of the activation cascade depends on the initiator and the microenvironment. Complement activation triggered by microbial infections culminates in the formation of membrane attack complexes to lyse the targets. Activation products of complement also serve as anaphylatoxins that activate and attract inflammatory cells, including neutrophils and macrophages to the site of complement activation. In rheumatic diseases, complement proteins play dual roles in the disease onset and pathogenesis. On the one hand, complement proteins are engaged in the prevention of autoimmunity, and a genetic deficiency of a *classical* pathway complement component proteins such as C1q, C1r, C1s, or C4 is among the strongest risk factors for systemic autoimmune disease such as SLE. Low levels of these complement proteins, which may be genetically determined or acquired, are frequently observed in SLE patients. On the other hand, complement activation is integral to tissue injuries and pathogenesis of established autoimmune disease or inflammatory disease, such as rheumatoid arthritis. There are ongoing research efforts to inhibit complement activation to ameliorate complement-mediated tissue injuries in inflammatory rheumatic diseases.

Cross-References

- ▶ Autoantibodies in Rheumatoid Arthritis
- ▶ Complement Regulation in the Kidney
- ▶ Discoid Lupus
- ▶ Discoid SLE
- ▶ Immunodeficiency in Autoimmune Diseases
- ▶ Juvenile Diseases: SLE in Children
- ▶ Rheumatoid Arthritis, Clinical Features
- ▶ Rheumatoid Arthritis, Genetics
- ▶ Skin in Systemic Lupus Erythematosus

▶ Systemic Lupus Erythematosus, Genetics
▶ Systemic Lupus Erythematosus, Pathogenesis
▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Agnello V. Lupus diseases associated with hereditary and acquired deficiencies of complement. Springer Semin Immunopathol. 1986;9:161–78.

Atkinson JP, Yu CY. Genetic susceptibility and class III complement genes. In: Systemic lupus erythematosus. Amsterdam: Elsevier Academic; 2011. p. 21–45.

Carroll MC, Isenman DE. Regulation of humoral immunity by complement. Immunity. 2012;37:199–207.

Chung EK, Wu YL, Yang Y, Zhou B, Yu CY. Human complement components C4A and C4B genetic diversities: complex genotypes and phenotypes. Curr Protoc Immunol. 2005;3:13.8.1–13.8.36.

Holers VM. Phenotypes of complement knockouts. Immunopharmacology. 2000;49:125–31.

Jensenius JC. The mannan-binding lectin (MBL) pathway of complement activation: biochemistry, biology and clinical implications. Adv Exp Med Biol. 2005;564:21–2.

Klos A, Wende W, Monk PM. International Union of Pharmacology. LXXXVII. Complement peptide C5a, C4a, and C3a receptors. Pharmacol Rev. 2013; 65: 500–43.

Korb L, Ahearn JM. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. J Immunol. 1997;158:4525–8.

Law SKA, Reid KBM. Complement. Oxford: IRL Press at Oxford University Press;1995.

Lipsker D, Hauptmann G. Cutaneous manifestations of complement deficiencies. Lupus. 2010;19:1096–1106.

Liu CC, Kao AH, Hawkins DM, Manzi S, Sattar A, Wilson N, Ahearn JM. Lymphocyte-bound complement activation products as biomarkers for diagnosis of systemic lupus erythematosus. Clin Transl Sci. 2009;2:300–8.

Lu JH, Teh BK, Wang L, Wang YN, Tan YS, Lai MC, Reid KB. The classical and regulatory functions of C1q in immunity and autoimmunity. Cell Mol Immunol. 2008;5:9–21.

Lv Y, He S, Zhang Z, Li Y, Hu D, Zhu K, Cheng H, Zhou F, Chen G, Zheng X, Li P, Ren Y, Yin X, Cui Y, Sun L, Yang S, Zhang X. Confirmation of C4 gene copy number variation and the association with systemic lupus erythematosus in Chinese Han population. Rheumatol Int. 2012;32:3047–53.

McClellan J, King MC. Genetic heterogeneity in human disease. Cell. 2010;141:210–7.

McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365:2205–19.

Okroj M, Heinegard D, Holmdahl R, Blom AM. Rheumatoid arthritis and the complement system. Ann Med. 2007;39:517–30.

Pickering MC, Botto M, Taylor PR, Lachmann PJ, Walport MJ. Systemic lupus erythematosus, complement deficiency and apoptosis. Adv Immunol. 2001;76:227–324.

Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11:785–97.

Rigby WF, Wu YL, Zan M, Zhou B, Rosengren S, Carlson C, Hilton W, Yu CY. Increased frequency of complement C4B deficiency in rheumatoid arthritis. Arthritis Rheum. 2012;64:1338–44.

Sim E, Gill EW, Sim RB. Drugs that induce systemic lupus erythematosus inhibit complement component C4. Lancet. 1984;ii:422–4.

Sturfelt G, Truedsson L. Complement in the immunopathogenesis of rheumatic disease. Nat Rev Rheumatol. 2012;8:458–68.

Taylor PR, Carugati A, Fadok VA, Cook HT, Andrews M, Carroll MC, Savill JS, Henson PM, Botto M, Walport MJ. A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. J Exp Med. 2000;192:359–66.

Tsao BP, Wu H. The genetics of human lupus. In: Dubois' lupus erythematosus. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 54–81.

Walport MJ. Complement-part I. New Engl J Med. 2001a;344:1058–66.

Walport MJ. Complement. Second of two parts. N Engl J Med. 2001b;344:1140–4.

Wu YL, Higgins GC, Rennebohm RM, Chung EK, Yang Y, Zhou B, Nagaraja N, Birmingham DJ, Rovin BH, Hebert LA, Yu CY. Three distinct profiles of serum complement C4 proteins in pediatric systemic lupus erythematosus (SLE) patients: tight associations of complement C4 and C3 protein levels in SLE but not in healthy subjects. Adv Exp Med Biol. 2006;586:227–47.

Yang Y, Chung EK, Wu YL, Savelli SL, Nagaraja HN, Zhou B, Hebert M, Jones KN, Shu Y, Kitzmiller K, Blanchong CA, McBride K, Higgins GC, Rennebohm RM, Rice RR, Hackshaw KV, Roubey RA, Grossman JM, Tsao BP, Birmingham DJ, Rovin BH, Hebert LA, Yu CY. Gene copy number variation and associated polymorphisms of complement component C4 in human systemic erythematosus (SLE): low copy number is a risk factor for and high copy number is a protective factor against European American SLE disease susceptibility. Am J Hum Genet. 2007;80:1037–54.

Yu CY, Hauptmann G, Yang Y, Wu YL, Birmingham DJ, Rovin BH, Hebert LA. Complement deficiencies in human systemic lupus erythematosus (SLE) and SLE nephritis: epidemiology and pathogenesis. In: Systemic lupus erythematosus: a companion to rheumatology. Philadelphia: Elsevier; 2007. p. 203–13.

Zhou W. The new face of anaphylatoxins in immune regulation. Immunobiology. 2012;217:225–34.

Complement Regulation in the Kidney

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Definition

The complement system maintains homeostasis by achieving a balance between complement activation and complement regulation. Under normal conditions this process is tilted towards regulation through mechanisms involving specific complement regulatory proteins. Even under conditions where complement activation is beneficial, such as for clearing immune complexes and contributing to the immune response, complement control must be maintained to prevent damage to bystander tissue. This is especially important in the kidney, where many pathologies involve the complement system.

Regulation of Complement Activation

The complement system comprises proteins involved in complement pathway activation, in regulation of this activation, and in receptors that mediate many of complement's functions (Walport 2001a, b). Complement activation can proceed by three different pathways, all of which yield enzymatic complexes (C3 convertases) that cleave C3 to C3a and C3b. The three activation pathways differ in how they are initiated. The classical pathway is initiated by fixation of C1q to appropriate substrates, such as immune complexes and apoptotic debris. The lectin pathway is initiated by the binding of mannose-binding lectin (MBL) or ficolin to carbohydrates found on the surface of certain pathogens. The alternative pathway is initiated spontaneously by a hydrolyzed form of C3 ($C3_{H20}$) that is continually present in the circulation and by C3b. Thus, the alternative pathway is always poised to be activated and is usually engaged to amplify complement activation by C3b generated by any of the pathways. Under normal circumstances, complement activation is held in check by regulatory systems.

Complement regulation involves both the natural decay of the C3 convertases as well as the actions of specific complement regulators (Zipfel and Skerka 2009). Perhaps the most important of these proteins are the regulators of complement activation (RCA), a family of proteins encoded in a cluster on chromosome 1 (Hourcade et al. 1989). All of the RCA proteins bind cleaved forms of C4 and/or C3, and through this action provide two general complement regulatory functions. First, this binding accelerates the decay of the convertases, which effectively halts activation of C3 and downstream complement effects (e.g., generation of C5a and membrane attack complex (MAC)). Second, this binding provides cofactor activity for the factor I-mediated cleavage of C4b (to C4d and C4c), C3b (to C3bi), and C3bi (to C3dg and C3c), forms that can no longer participate in convertase activity. Factor I (FI) is a serine esterase with specificity for C4b, C3b, and C3bi. Through these two general functions, the RCA proteins prevent spontaneous complement activation and keep amplified complement activation in check.

There are six general types of RCA proteins, each with its own combination of function and distribution (Table 1). *C4b-binding protein* (*C4BP*) is a plasma protein that binds C4b and provides both decay acceleration activity for the classical pathway convertases and cofactor activity for the FI-mediated cleavage of C4b. *Factor H* (*FH*) is a plasma protein that binds C3b and provides both decay acceleration activity for the alternative pathway convertases and cofactor activity for the FI-mediated cleavage of C3b. There are actually seven different forms of FH from six different genes (FH, FH-like protein 1 (an alternative splice product of FH), FH-related protein 1 (FHR1), FHR2, FHR3, FHR4, and FHR5) (Rodriguez de Cordoba et al. 2004). The predominant form in the plasma is FH, and the unique physiological roles of FH-like protein

Complement Regulation in the Kidney, Table 1 The regulators of complement activation

RCA protein	Ligand	Complement regulatory function		Predominant site of activity
		Decay acceleration	Factor I cofactor	
C4BP	C4b	+	+	Fluid phase
FH	C3b	+	+	Fluid phase, membrane
MCP	C3b, C4b	–	+	Membrane (intrinsic)
DAF	C3 convertase	+	–	Membrane (intrinsic)
CR1	C3b, C4b, C3bi	+	+	Membrane (intrinsic and extrinsic)
CR2	C3bi, C3dg	–	+[a]	Membrane

[a]Cofactor activity only for cleavage of C3bi to C3dg

1 and the related FH gene products are presently unclear. *Membrane cofactor protein* (*MCP, CD46*) is a type 1 membrane protein that binds to and provides cofactor activity for the FI-mediated cleavage of both C3b and C4b. *Decay accelerating factor* (*DAF, CD55*) is a glycophosphatidylinositol (GPI)-linked membrane protein that interacts with all of the complement convertases and accelerates their decay. This interaction is unique among the RCA proteins in that it occurs through the recognition of the assembled convertase, rather than through the individual components. *The complement receptor type 1* (*CR1, CD35*) is a type 1 membrane protein that binds to and provides cofactor activity for the FI-mediated cleavage of C3b, C3bi, and C4b and accelerates the decay of all of the convertases. The sixth RCA protein, *the complement receptor type 2* (*CR2, CD21*), is a receptor for C3bi and C3dg and has demonstrated mild cofactor activity for cleavage of C3bi. However, the physiological role of CR2 is likely not as a complement regulator and will not be discussed further in this entry.

In addition to differences in how they regulate complement, the RCA proteins also differ in the tissue compartment in which they provide this function. As plasma proteins, C4BP and FH are important in regulating fluid-phase complement activation (e.g., in plasma). FH also have important complement regulatory function at specific tissue sites, through binding sites for C-reactive protein (CRP), heparin, and sialic acid. The membrane proteins MCP and DAF exert their complement regulation locally at the membrane surface, specifically on the same membrane where they are expressed ("intrinsic" function). The membrane protein CR1 exhibits both intrinsic activity and extrinsic activity, being able to regulate complement activation in the fluid phase as well as on external surfaces. The extrinsic binding of C3b, C3bi, and C4b by CR1 in the circulation that leads to complement regulation occurs mainly through erythrocyte CR1, and this binding is referred to as immune adherence (Birmingham and Hebert 2001).

Other non-RCA complement regulators also play important roles in complement regulation, including C1 inhibitor (C1INH) and CD59. C1INH is a soluble serine protease inhibitor that functions by binding activated C1r and C1s, causing their dissociation from the C1q molecule and preventing initiation of the classical pathway. CD59 is a GPI-linked membrane protein that binds to C8/C9 during the process of MAC formation, thus halting it, and in so doing protects membranes from the damaging effects of MAC.

Complement Regulation in the Kidney

All tissues are vulnerable to damage through complement activation. Under normal conditions, this damage is prevented by the action of the complement regulators in the blood and complement regulators on tissue exposed to blood. However, during appropriate complement activation by substrates such as microorganisms or immune complexes, some of the resulting complement effector proteins (e.g., MAC) can spillover and cause damage to nearby host tissue. The kidney appears to be particularly vulnerable to this type of complement-mediated damage.

As a natural filter and under high pressure and turbulence, the kidney tends to trap the types of debris that can activate complement. An example of this is the trapping of immune complexes that result following infection or the development of autoimmunity involving autoantibodies (e.g., lupus nephritis, LN). In LN, classical complement activation by immune complexes that have accumulated in the kidney tissue can initiate damage to the tissue. Alternative pathway complement activation, either amplified as a result of the initial classical pathway or directly activated through damaged tissue, perpetuates the complement-mediated damage.

Perhaps as an evolutionary response to the heightened vulnerability to complement activation, complement regulatory mechanisms exist that are specific to the kidney. For example, CR1 is expressed at high levels in glomerular podocytes (Emancipator et al. 1983). The function of glomerular podocyte CR1 is currently unproven, though this expression site likely provides complement regulation targeted to the glomerular filtration barrier, thus suppressing complement activation in the event that immune complexes become trapped at this barrier or that the filtration barrier is disrupted. In support of this, loss of podocyte CR1 commonly occurs in patients with lupus nephritis or diffuse diabetic nephropathy.

Another RCA expression pattern specific to the kidney involves DAF and MCP, the two RCA proteins that are important for intrinsic complement regulation on tissue surfaces. For DAF, normal human kidney expression is limited to the juxtaglomerular apparatus (JGA). In certain diseased kidneys, such as those from patients with LN or with hemolytic uremic syndrome (HUS)-related kidney damage, DAF expression is lost from the JGA and becomes prominent in the glomerular mesangium and interstitium and correlates with C3 deposition (Cosio et al. 1989). Normal kidney MCP expression is similar to DAF in that it is predominantly in the JGA, though weak expression also occurs at many other sites, such as basement membranes and the mesangium. In contrast to DAF, MCP expression does not change in the JGA in LN (or in membranoproliferative glomerulonephritis, membranous nephropathy (MN), and poststreptococcal acute glomerulonephritis), but its expression is greatly increased on the glomerular capillary wall and in the mesangium (Endoh et al. 1993). Similar to DAF, the levels of MCP correlate with the levels C3b/C3bi deposition. These findings suggest that DAF and MCP expression is increased in the kidney in response to complement activation during disease processes. The relevance of DAF and MCP localization within the JGA under normal healthy conditions remains unclear, though it may be part of the normal JGA response to common changes in glomerular filtration rate and renal blood flow.

Though not specifically expressed in the kidney, FH may also play a particularly important role in protecting the kidney from complement-mediated damage. As mentioned above, though often thought of as one of the circulating regulatory proteins, FH also has binding sites to heparin, sialic acid, and CRP. These binding sites may target FH to certain tissue and, in the case of the CRP binding site, may target FH to sites of tissue damage, thus targeting complement regulation in a region where complement activation may be ongoing. The importance of this has recently been recognized in studies involving the disease age-related macular degeneration (AMD). A common polymorphism in the FH gene causes a tyrosine (Y) to histidine (H) substitution at amino acid position 402, which falls in the same region of the CRP binding site. The 402H variant exhibits reduced FH binding to CRP and reduced targeted complement regulation. The 402H variant is associated with AMD and in fact imparts ~50 % risk for AMD. Together, these data suggest that the developing lesion in AMD is driven in large part by complement activation in the face of dysfunctional complement regulation by FH. A similar relationship was recently found with LN, where the 402H variant was associated with complement activation during LN flare (Birmingham et al. 2010). Thus, FH may play a significant role in complement regulation in the kidney of lupus nephritis patients during disease flare.

As discussed above, complement activation needs to be tightly regulated to protect self-tissue, even in the absence of an overt activating insult, and complement regulators provide this function. This role appears to be particularly important for general kidney health. The best evidence of this can be found in patients with dense deposit disease (DDD), also known as membranoproliferative glomerulonephritis (MPGN) type II. Patients with this disease have defects that are either related to C3NeF (~80 % of cases), which is an autoantibody to the alternative pathway C3 convertase that stabilizes the convertase, or to FH/FHR protein defects (mutations, deletions) (Smith et al. 2011). In both cases, spontaneous and prolonged alternative pathway complement activation occurs that leads to complement deposition in the renal vasculature and eventual accumulation of complement-containing dense deposits in the glomerular basement membrane (GBM). Clinical manifestations of this defect include proteinuria, hypertension, and progression to end-stage kidney disease (ESKD). Thus, the vasculature in the kidney appears to be a prime target for spontaneous complement activation due to dysfunctional complement regulation.

A similar disease has been recently described, termed complement FHR5 (CFHR5) nephropathy (Gale et al. 2010). This familial disease was discovered in two Cyprus families and was found to be associated with a variant of the FHR5 gene that exhibits reduced binding to deposited C3b. The clinical presentation included proteinuria, hematuria, and ESKD and coincided with the appearance of C3 deposition and electron-dense deposits in the capillary wall and mesangium. This is an intriguing finding from the standpoint that circulating levels are <10 % of the level of FH and suggests that FHR5 provides complement regulatory effects that are specific to the kidney.

Another form of renal disease that is directly linked to defective complement regulation is atypical HUS (aHUS), which is manifested by hemolytic anemia, thrombocytopenia, and acute renal failure from vascular thrombosis, in the absence of enteropathic infection (usually *Escherichia coli*). The majority of aHUS cases appear to be associated with deleterious mutations in the complement regulatory proteins FH, MCP, and FI, with a penetrance of about 50 % (Richards et al. 2007). Interestingly, patients with aHUS often have mutations in more than one regulatory protein, and onset of the disease is variable, suggesting this disease requires two hits, such as multiple regulatory defects or an environmental contribution (e.g., infection), for full disease onset. In the case of an environmental factor such as infection, this second hit likely initiates complement activation, which is prolonged in the absence of adequate complement regulation, resulting in complement-mediated vascular damage in the kidney. Such a scenario suggests that inefficient complement regulation may also contribute to renal vascular injury associated with typical HUS involving E. coli infections (Orth and Wurzner 2010).

Conclusions

The purpose of complement regulation is to control spontaneous complement activation and to keep appropriate complement activation in check, thus maintaining adequate complement levels and preventing complement-mediated damage to self-tissue. This is especially important to the kidney, an organ which bears the brunt of inappropriate or uncontrolled complement activation. Complement regulatory mechanisms exist that are unique to the kidney to provide enhanced protection against complement-mediated damage. Defects in these complement regulatory mechanisms can directly lead to kidney injury, and secondarily have a negative impact on kidney diseases that involve the complement system.

Cross-References

- ▶ Complement in Rheumatic Diseases
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Birmingham DJ, Hebert LA. CR1 and CR1-like: the primate immune adherence receptors. Immunol Rev. 2001;180:100–11.

Birmingham DJ, Irshaid F, Nagaraja HN, Zou X, Tsao BP, Wu H, Yu CY, Hebert LA, Rovin BH. The complex nature of serum C3 and C4 as biomarkers of lupus renal flare. Lupus. 2010;19: 1272–80.

Cosio FG, Sedmak DD, Mahan JD, Nahman Jr NS. Localization of decay accelerating factor in normal and diseased kidneys. Kidney Int. 1989;36: 100–7.

Emancipator SN, Iida K, Nussenzweig V, Gallo GR. Monoclonal antibodies to human complement receptor (CR1) detect defects in glomerular diseases. Clin Immunol Immunopathol. 1983;27:170–5.

Endoh M, Yamashina M, Ohi H, Funahashi K, Ikuno T, Yasugi T, Atkinson JP, Okada H. Immunohistochemical demonstration of membrane cofactor protein (MCP) of complement in normal and diseased kidney tissues. Clin Exp Immunol. 1993;94:182–8.

Gale DP, de Jorge EG, Cook HT, Martinez-Barricarte R, Hadjisavvas A, McLean AG, Pusey CD, Pierides A, Kyriacou K, Athanasiou Y, Voskarides K, Deltas C, Palmer A, Fremeaux-Bacchi V, de Cordoba SR, Maxwell PH, Pickering MC. Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. Lancet. 2010;376:794–801.

Hourcade DE, Holers VM, Atkinson JP. The regulators of complement activation (RCA) gene cluster. Adv Immunol. 1989;45:381–416.

Orth D, Wurzner R. Complement in typical hemolytic uremic syndrome. Semin Thromb Hemost. 2010;36: 620–4.

Richards A, Kavanagh D, Atkinson JP. Inherited complement regulatory protein deficiency predisposes to human disease in acute injury and chronic inflammatory states. The examples of vascular damage in atypical hemolytic uremic syndrome and debris accumulation in age-related macular degeneration. Adv Immunol. 2007;96:141–77.

Rodriguez de Cordoba S, Esparza-Gordillo J, Goicoechea de Jorge E, Lopez-Trascasa M, Sanchez-Corral P. The human complement factor H: functional roles, genetic variations and disease associations. Mol Immunol. 2004;41:355–67.

Smith RJ, Harris CL, Pickering MC. Dense deposit disease. Mol Immunol. 2011;48:1604–10.

Walport MJ. Complement. First of two parts. N Engl J Med. 2001a;344:1058–66.

Walport MJ. Complement. Second of two parts. N Engl J Med. 2001b;344:1140–4.

Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. Nat Rev Immunol. 2009;9: 729–40.

CTLA-4

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Synonyms

APC- antigen presenting cell; ARF- ADP-ribosylation factor; CD- clusters of differentiation; cdk- cyclin-dependent kinases; CTLA- 4 – cytotoxicT lymphocyte antigen 4; ICOS- Inducible T-cell CoStimulator; IDO - indoleamine 2, 3-dioxygenase; ITIM -immunoreceptor tyrosine-based inhibitory motif; LAT- linker of activated T cells; LAX - linker for activation of X cells; LFA-1 – lymphocyte function-associated antigen-1; MHC- major histocompatibility complex; PLD- phospholipase D; PP2A- serine/threonine phosphatase 2A; SIT - SHP2-interacting transmembrane adapter protein; SLE- Systemic Lupus Erythematosus; T reg – regulatory T cell; TCR- T cell antigen receptor; TIRC7- T cell immune response cDNA *7*; TLR- Toll-like receptor; TRIM - T-cell receptor interacting molecule; ZAP-70 -Zeta-chain-associated protein kinase 70

Definition

For antigen-induced activation of a T cell to occur, an antigen-presenting cell (APC) must provide the T cell with two signals. The first is the antigen, in the context of the major histocompatibility complex (MHC) on the surface of the APC (“a hot dog [the antigen] in a hot dog bun [the groove provided by the MHC molecules]”). The second is the costimulatory signal, classically delivered by CD80/CD86 (B7-1/B7-2; members of the immunoglobulin superfamily consisting of variable and constant domains with approximately 20 % sequence identity with each other) on the APC interacting with CD28 (another

immunoglobulin superfamily member, consisting of a disulphide bond-linked homodimer) on the surface of the T cell (Linsley et al. 1991). CD28 is constitutively expressed on the surface of T cells; the receipt of both "signal 1," antigen in the context of MHC, and "signal 2," the costimulatory signal, allows antigen-specific activation of the T cell. Absence of "signal 2" causes the T cell to be anergized. CD80/86 expression on the surface of APCs is upregulated by exposure of the APC to "danger" signals, e.g., ligands of toll-like receptors (TLRs), making the APC a more potent activator of T cells (Hertz et al. 2001).

Once optimal antigen activation occurs, there is a need for a negative signal, a means to dampen T cell activation; theoretically, the absence of this inhibitory signal could lead to excessive expansion of the T cell population, with uncontrolled lymphoproliferation and/or poorly modulated immune function, perhaps resulting in autoimmune reactivity. Thus, there is a need for a "yin" to offset the "yang" of "signal 2" provided by the binding of CD80/CD86 to CD28.

CTLA-4 (cytotoxic T lymphocyte antigen 4) was the fourth in a series of molecules identified on the surface of cytotoxic T lymphocytes, a molecule at first in search of a function, until its "yin"-like qualities were identified in the early 1990s (Noel, Boise, Thompson, 1996). And, as predicted, mice unable to express CTLA-4 were predisposed to lethal lymphoproliferative disorders.

CTLA-4 (CD152): Function and Mechanism of Action

CTLA-4 (CD152), like CD28, is a member of the immunoglobulin superfamily, a disulphide bond-linked homodimer with approximately 30 % sequence identity of its variable Ig-like chain with the analogous CD28 chain. Of note, and predictably, CTLA-4 is not expressed on resting T cells prior to or early in the process of T cell activation by the two signals from the APC. Presynthesized CTLA-4 is stored within intracellular vesicles, including the *trans*-Golgi network, overlapping with endocytic compartments and with perforin-containing secretory granules. With increasing intracellular calcium levels that follow T cell activation, CTLA-4 is upregulated on the surface of the activated T cell during cell-cycle progression, under the control of several signaling molecules, such as:

TRIM [TCR-interacting molecule], which acts as a chaperone

LAX and SIT [linker for activation of X cells and SHP2-interacting transmembrane adapter protein, respectively], either of which can substitute for TRIM

PLD and ARF-1 [phospholipase D and adenosine diphosphate-ribosylation factor-1, respectively] needed for budding of vesicles at the Golgi apparatus

TIRC7 [T cell immune response cDNA 7], which is colocalized with CTLA-4 on activated T cells and delivers an apoptotic signal to CD4+ and CD8+ T cells after it interacts with its ligand, the non-polymorphic alpha 2 domain of HLA-DR, HLA-DRα2

The short surface half-life of CTLA-4 suggests tight regulation of its surface expression. Both intracellular and cell surface CTLA-4 are focused on sites of T cell antigen receptor activation, which is to say the "immunological synapse" (Linsley, Brady, Greene, et al. 1996). Recent work shows localization of CTLA-4 to the central supramolecular synapse, where it becomes stabilized by its interaction with its ligands (CD80/86), while CD28 is excluded from interaction with CD80/86 (Yokosuka et al. 2010). Although CD80 and CD86 express only 20 % of overall amino acid identity, they both bind CD28 and CTLA-4. The latter is the high-avidity receptor for both CD80 and CD86, binding approximately 500- to 2,500-fold more avidly to CD80 and CD86 than does CD28. Thus, CTLA-4 "outcompetes" CD28 for binding with CD80/CD86; CTLA-4 then transmits an inhibitory signal to the T cell, balancing out the previously stimulatory signal of CD28 (Stamper et al. 2001).

The effects of CTLA-4 have been divided into "cell-autonomous" and "non-cell-autonomous" effects. The former represents CTLA-4

acting as an inhibitory coreceptor in T cells expressing CTLA-4, whereas the latter represents CTLA-4 on one T cell inhibiting activities or functions of other T cells. Non-cell-autonomous suppression of T cell activation may be accomplished by a regulatory population of T cells, known as Tregs; details of this phenomenon are described below.

The initial theory about how CTLA-4 delivers its inhibitory signal to the activated T cell was that CTLA-4 "outcompeted" CD28 for binding to CD80/86, resulting in a simple loss or withdrawal of the stimulation provided by CD28 signaling – this then caused the T cell to slow its activation. However, there are other reasonable explanations. Englehardt and colleagues (Englehardt et al. 2006) suggested five potential explanations:

1. As above, CTLA-4 outcompetes CD28, removing that stimulatory signal.
2. CTLA-4 delivers a negative stimulus of its own, signaling through its own intracytoplasmic tail, affecting CD28 and/or the T cell antigen receptor (TCR); this could be mediated by a number of established CTLA-4 activities, e.g., reduction of IL-2 production, decreased expression of cyclin D3, cyclin- dependent kinases (cdks) 4 and 6, or recruitment of SHP2 and serine/threonine phosphatase 2A (PP2A) to the immunoreceptor tyrosine-based inhibitory motif (ITIM) in CTLA-4's cytoplasmic tail, leading to dephosphorylation of TCR- proximal signaling proteins such as CD3ξ and ▶ LAT (linker for activated T cells), and possibly Shc, Lck, Fyn, and ZAP-70 (zeta-chain-associated protein kinase 70). CTLA-4 interferes with the formation of micro-clustering of ZAP-70, which is needed for transmission of the TCR signal (Rudd et al. 2009). Part of this negative signaling may be due to interference with the production of IL-2.
3. CTLA-4 may interfere with the integrity of the immunological synapse, by sterically interfering with tight fit, or by mechanisms described above.
4. CTLA-4 may have an effect on, or be part of, the functioning of regulatory T cells.
5. CTLA-4 may have an indirect effect by suppressing APC function, e.g., induction of indoleamine 2, 3-dioxygenase (IDO) within the APC that, by depleting local tryptophan, can inhibit T cell activation; there may be decreases in CD80/86 expression by endocytosis or trogocytosis of CTLA-4-CD80/86 complexes by Tregs; soluble CTLA-4 may inhibit access of CD28 to CD80/86.

Their study, published in 2006, shows that the effect of CTLA-4 is dependent on the presence of CD28 and supports the first two potential mechanisms (Englehardt et al. 2006). Rudd et al. described yet another mechanism: CTLA-4 interferes with the TCR signal that slows the migrating T cell, allowing it to halt and form a stable immunological synapse (Schneider et al. 2006).

Madrenas and colleagues (Madrenas et al. 2004) suggest that prior to binding CD80/86, CTLA-4 may exist in two inactive states. In one, PP2A cannot gain access to the cytoplasmic tail of CTLA-4, which is folded on the inner leaflet of the cell membrane; in the other CTLA-4 state, the cytoplasmic tail is accessible to PP2A. When CTLA-4 is colligated with the TCR, the PP2A is phosphorylated and then dissociates from the CTLA-4, unleashing its inhibitory signaling potential.

As noted by Rudd et al. in their review, CTLA-4 not only activates JNK in CD4+ T cells but it inhibits ERKs (extracellular signal-regulated kinases), thus differentially affecting various members of the greater MAPK (mitogen-activated protein kinase) family. Activation of JNK could influence Th1 over Th2 differentiation and enhance LFA-1 adhesion, among other changes (Rudd et al. 2009).

CTLA-4: Structure

CTLA-4 includes an extracellular V domain, a transmembrane domain, and a cytoplasmic tail, with the intracellular domain being similar to that of CD28, in that neither have catalytic activity. The membrane-bound isoform of CTLA-4 functions as a homodimer with a single

connecting disulfide bond, while the soluble isoform is a monomer.

Three isoforms of CTLA-4 have been identified. The full-length isoform (flCTLA-4) is expressed only on activated T cells. A form lacking the ectodomain (CTLA-4i) is expressed only on resting T cells. The third form is a soluble CTLA-4 (sCTLA-4) which lacks the transmembrane section encoded by exon 3; it is not clear to what degree sCTLA-4 is active, e.g., in modulating normal immune function and/or defending against the elaboration of an autoimmune process, although elevated levels of sCTLA-4 have been identified in patients with autoimmune diseases (Rudd et al. 2009; Park, Baek, Do, et al., 2009).

The intracellular component of CTLA-4 contains one YVKM motif capable of binding SHP2, PP2A, and/or ▶ PI3K and a single proline-rich motif which can bind SH3-containing proteins. CTLA-4 inhibits T cell responses, but there is still uncertainty about how this inhibitory signal is delivered.

CTLA-4 and Regulatory T Cells

CTLA-4 has been implicated in the maintenance of peripheral T cell tolerance, the avoidance of autoimmunity. One of the crucial processes involved in this maintenance of peace is immune suppression by a family of $CD4^+$ $CD25^+$ (IL-2 receptor α-chain) $FOXP3^+$ T cells called natural regulatory T cells, or Tregs, that inhibit T cell activation. This T cell population constitutively expresses CTLA-4 on its surface, in contradistinction to naïve T cells early in their activation by APCs; expression of CTLA-4 by Tregs increases after TCR ligation, as is the case with standard antigen-specified T responder cells. As noted, some of the actions of CTLA-4 on the Tregs constitute the "cell-non-autonomous" actions alluded to above (Wing et al. 2011).

As reviewed by Friedline et al. (2009), transfer of naive ($CD25^-$) $CD4^+$ T cells into lymphopenic hosts leads to colitis, which is blocked if $CD4^+CD25^+$ $FOXP3^+$ Treg cells are also included in the transfer. This protection from colitis is in turn blocked by injection of antibodies to CTLA-4, which supports the premise that CTLA-4 on Treg is important in this immune modulation. Friedline and colleagues recently demonstrated that regulation is mediated by CTLA-4 Treg cells; is reversible, depending on the continuous presence of these Treg cells; and does not involve "reverse inside-out" B7 signaling.

Tang and colleagues demonstrated that expression of CTLA-4 on $FOXP3^+$ Tregs in vivo is related to their rapid, perpetual homeostasis and that upregulation of CTLA-4 occurs solely on $FOXP3^+$ Tregs undergoing extensive proliferation, which can be reversed by inhibiting the CD28 pathway, with subsequent reduction in $FOXP3^+$ Treg proliferation and frequency (Tang et al. 2008). FOXP3 represses IL-2 production and that of other proinflammatory cytokines and increases the expression of both CD25 and CTLA-4. These cells also express high levels of lymphocyte function-associated antigen-1 (LFA-1), perhaps helping to explain why Tregs outcompete conventional T cells for aggregation around APCs, essentially physically preventing responder T cells access to a forming immunological synapse. CTLA-4 negatively regulates steady-state Treg homeostasis; when CTLA-4 signaling is inhibited, Treg proliferation and overall Treg frequency is enhanced.

Thus, there is evidence that CTLA-4 on the surface of Tregs is serving a homeostatic function for that population of T cells, although it almost certainly plays a direct role in the regulatory pathway by suppressing APCs (Friedline et al. 2009). CTLA-4 may also have an important role in cell survival. CTLA-4 may have the ability to reverse antigen-induced cell death in anergic T cells, perhaps by known effects on various members of the Bcl-2 protein family (Rudd et al. 2009).

A recent brief report by Ying et al. is the first demonstration that CTLA-4 has an effect on Th17 cell function. A CTLA-4 interaction with B7 inhibits Th17 cell differentiation in vitro and suppresses the development of Th17-mediated autoimmunity in vivo, utilizing an autoimmune myocarditis model (Ying et al. 2010).

CTLA-4 Polymorphisms and Linkage to Immune-Mediated Diseases/ Susceptibility to Diseases

Alternate splice variants of CTLA-4, encoding different isoforms, have been identified, as noted above. Mutations in the CTLA-4 gene have been associated with insulin-dependent diabetes mellitus, Graves' disease, Hashimoto's thyroiditis, celiac disease, ▶ systemic lupus erythematosus (SLE), thyroid-associated damage of the orbital contents ("ophthalmopathy"), ▶ primary biliary cirrhosis, and other ▶ autoimmune diseases.

Polymorphisms of the CTLA-4 gene are associated with autoimmune diseases such as autoimmune thyroid disease and multiple sclerosis, though this association is often weak. In systemic lupus erythematosus, the splice variant sCTLA-4 is found to be aberrantly produced and found in the serum of patients with active disease.

CTLA-4 variants have been implicated in immune-mediated, infectious, and malignant diseases, both as risk/susceptibility factors and as correlates of decreased risk/severity (as summarized in Sigal 2012). Some of the disease associations include:

Certain forms of CTLA-4 are protective and others connote susceptibility in Japanese patients with biliary cirrhosis.

Among many genes examined in a Chinese study, one variant of CTLA-4 represents a risk factor for breast cancer.

Polymorphisms of CTLA-4 and CD86 represent possible factors associated with risk or protection for chronic obstructive pulmonary disease in this Chinese population study.

CTLA-4 variants have been implicated in a genome-wide search for innate and adaptive immune risk factors for the development of alopecia areata, among the most prevalent of human autoimmune diseases.

CTLA-4 variants have been implicated in rheumatoid arthritis, SLE, Behcet's syndrome, and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). A deficiency (production or maintenance) of regulatory T cells in SLE patients may be due to variants in CTLA-4 and/or FOXP3.

In a study of Estonian and Finnish patients with type 1 diabetes mellitus, CTLA-4 but not inducible T cell costimulator (ICOS; CD278) gene variants were implicated in susceptibility to type 1 diabetes mellitus. The alleles connoting risk were more common in the Finnish controls compared with the Estonians, suggesting this gene locus might also contribute to the higher disease incidence in Finland.

CTLA-4 as a Therapeutic Target

The ability of CTLA-4 to modify T cell function has made it an obvious therapeutic target: antagonism for suppression of autoimmune responses or graft rejection and agonism to enhance immune responses to malignancy.

In the former category is the first such "costimulation modulator," abatacept (CTLA-4-Ig), consisting of the extracellular domain of CTLA-4 fused to a human IgG1 Fc backbone (hinge-CH2-CH3 domains), which had been modified to interfere with complement fixation (the purpose of the Fc component is to increase the serum half-life of the therapeutic agent). Abatacept, marketed as Orencia®, is approved for use in rheumatoid arthritis (Maxwell and Singh 2009) and juvenile idiopathic arthritis (Ruperto et al. 2008, 2010) and has been studied in psoriatic arthritis (Mease et al. 2011) and systemic lupus erythematosus (Merrill et al. 2010). A related molecule (two mutations different from abatacept) is belatacept, recently approved and marketed as Nulojix®, for use in renal transplantation as an alternative to cyclosporine (Vincenti et al. 2011). The effect of each of these drugs is to enhance CTLA-4-mediated suppression of ongoing immune responses in order to control autoimmune disorders (abatacept) and transplant graft rejection (belatacept). Although there had been speculation that abatacept might work, at least in part, by enhancing IDO in APCs, this was disproven by Davis et al. (Davis et al. 2008).

In the latter category is ipilimumab, a fully humanized IgG1 monoclonal antibody that

targets and blocks CTLA-4 on human cytotoxic cells. The result of this is to remove CTLA-4-induced suppression of these cytotoxic cells, allowing expansion of the activated clones, with a subsequent increase in antitumor activity. Previously known as MDX-010 or MDX-101, ipilimumab (Sondak et al. 2011) has been approved for use in metastatic melanoma and is now marketed as Yervoy®. Ipilimumab is still in trials for both small cell and non-small cell lung carcinoma, as well as for metastatic hormone therapy-refractory prostate cancer. Another fully human monoclonal (an IgG2) anti-CTLA-4 antibody is tremelimumab (formerly ticilimumab, CP-675,206) in trials for pancreatic, prostate, and bladder cancer; a recent study suggests this molecule has a direct impact on effector cells, rather than an indirect pathway leading through regulatory T cells (Khan et al. 2011).

Conclusion

In less than two decades, CTLA-4 has gone from a molecule in search of a purpose to one of the underpinnings of the newly described phenomenon of properly modulated "costimulation" and most recently a set of at least three successful therapeutics. As is so often the case, spontaneous "experiments of nature" have provided us with insights of how the presence of alternate forms of CTLA-4 or its absence alter normal immune function. The world of costimulation has become a bit more complex with the addition of other parallel alternate pathways to T cell activation.

Disclosure

Dr. Sigal was the medical lead at Bristol-Myers Squibb (BMS) for clinical trials of abatacept in juvenile idiopathic arthritis, psoriatic arthritis, and systemic lupus erythematosus. He is no longer employed by BMS, but owns a small amount of BMS stock.

Cross-References

- ▶ CD5
- ▶ CTLA4-Ig
- ▶ Regulatory B Cells
- ▶ Resolution of Inflammation
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver

References

Davis PM, Nadler SG, Stetsko DK, Suchard SJ. Abatacept modulates human dendritic cell-stimulated T-cell proliferation and effector function independent of IDO induction. Clin Immunol. 2008;126(1):38–47. Epub 2007 Oct 22.

Englehardt JJ, Sullivan TJ, Allison JP. CTLA-4 overexpression inhibits T cell responses through CD28-B7-dependent mechanism. J Immunol. 2006;177:1052–61.

Friedline RH, Brown DS, Nguyen H, Kornfeld H, Lee J, Zhang Y, Appleby M, Der SD, Kang J, Chambers CA. CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. J Exp Med. 2009;206(2):421–34. Epub 2009 Feb 2.

Hertz CJ, Kiertscher SM, Godowski PJ, Bouis DA, Norgard MV, Roth MD, Modlin RL. Microbial lipopeptides stimulate dendritic cell maturation via Toll-like receptor 2. J Immunol. 2001;166(4):2444–50.

Khan S, Burt DJ, Ralph C, Thistlethwaite FC, Hawkins RE, Elkord E. Tremelimumab (anti-CTLA4) mediates immune responses mainly by direct activation of T effector cells rather than by affecting T regulatory cells. Clin Immunol. 2011;138(1):85–96. Epub 2010 Nov 5.

Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med. 1991;174(3):561–9.

Linsley PS, Bradshaw J, Greene J, Peach R, Bennett KL, Mittler RS. Intracellular trafficking of CTLA-4 and focal localization towards sites of TCR engagement. Immunity. 1996;4(6):535–43.

Madrenas J, Chau LA, Teft WA, Wu PW, Jussif J, Kasaian M, Carreno BM, Ling V. Conversion of CTLA-4 from inhibitor to activator of T cells with a bispecific tandem single-chain Fv ligand. J Immunol. 2004;172:5948–56.

Maxwell L, Singh JA. Abatacept for rheumatoid arthritis. Cochrane Database of Syst Rev. 2009 (4):CD007277. doi:10.1002/14651858.CD007277.pub2.

Mease P, Genovese MC, Gladstein G, Kivitz AJ, Ritchlin C, Tak PP, Wollenhaupt J, Bahary O, Becker J-C,

Kelly S, Sigal L, Tang J, Gladman D. Abatacept in the treatment of patients with psoriatic arthritis: results of a double-blind, randomized, placebo-controlled phase 2 trial. Arthritis Rheum. 2011;63:939–48.
Merrill JT, Burgos-Vargas R, Westhovens R, Chalmers A, D'Cruz D, Wallace DJ, Bae SC, Sigal L, Becker J-C, Kelly S, Raghupathi K, Lit T, Peng Y, Kinaszczuk M, Nash P. The efficacy and safety of abatacept in systemic lupus erythematosus: the results of a 12 month exploratory study. Arthritis Rheum. 2010;62(10): 3077–87.
Noel PJ, Boise LH, Thompson CB. Regulation of T cell activation by CD28 and CTLA4. Adv Exp Med Biol. 1996;406:209–17.
Park KS, Baek JA, Do JE, Bang D, Lee ES. CTLA4 gene polymorphisms and soluble CTLA4 protein in Behcet's disease. Tissue Antigens. 2009;74(3):222–7. Epub 2009 Jun 25.
Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. Immunol Rev. 2009;229(1):12–26.
Ruperto N, Lovell DJ, Quartier P, Paz E, Rubio-Pérez N, Silva CA, Abud-Mendoza C, Burgos-Vargas R, Gerloni V, Melo-Gomes JA, Saad-Magalhães C, Sztajnbok F, Goldenstein-Schainberg C, Scheinberg M, Calvo Penades I, Fischbach M, Orozco J, Hashkes PJ, Hom C, Jung L, Lepore L, Oliveira S, Wallace C, Sigal LH, Block AJ, Covucci A, Martini A, Giannini EH, for the Paediatric Rheumatology INternational Trials Organization (PRINTO) and the Pediatric Rheumatology Collaborative Study Group (PRCSG). Efficacy and safety of abatacept in children with juvenile idiopathic arthritis: a randomized, double-blind, placebo-controlled withdrawal trial. Lancet. 2008;372(9636):383–91.
Ruperto N, Lovell DJ, Quartier P, Paz E, Rubio-Pérez N, Silva CA, Abud-Mendoza C, Burgos-Vargas R, Gerloni V, Melo-Gomes JA, Saad-Magalhães C, Chavez J, Huemer C, Kivitz A, Blanco FJ, Foeldvari I, Hofer M, Horneff G, Huppertz H-I, Job Deslandre C, Loy A, Minden K, Punaro M, Nunez AF, Sigal LH, Block AJ, Nys M, Martini A, Giannini EH, for the Paediatric Rheumatology INternational Trials Organization (PRINTO) and the Pediatric Rheumatology Collaborative Study Group (PRCSG). Long-term safety and efficacy of abatacept in children with juvenile idiopathic arthritis. Arthritis & Rheum. 2010;62(6):1792–802.
Schneider H, Downey J, Smith A, Zinselmeyer BH, Rush C, Brewer JM, Wei B, Hogg N, Garside P, Rudd CE. Reversal of the TCR stop signal by CTLA-4. Science. 2006;313(29 Sept):1972–5.
Sigal LH. Basic science for clinicians 55: CTLA-4. J Clin Rheumatol. 2012;18(3):155–8. doi:10.1097/RHU.0b013e31824ea103.
Sondak VK, Smalley KS, Kudchadkar R, Grippon S, Kirkpatrick P. Ipilimumab. Nat Rev Drug Discov. 2011;10(6):411–2.
Stamper CC, Zhang Y, Tobin JF, Erbe DV, Ikemizu S, Davis SJ, Stahl ML, Seehra J, Somers WS, Mosyak L. Crystal structure of the B7-1/CTLA-4 complex that inhibits human immune responses. Nature. 2001; 410(6828):608–11.
Tang AL, Teijaro JR, Njau MN, Chandran SS, Azimzadeh A, Nadler SG, Rothstein DM, Farber DL. CTLA4 expression is an indicator and regulator of steady-state CD4+ FoxP3+ T cell homeostasis. J Immunol. 2008;181(3):1806–13.
Vincenti F, Dritselis A, Kirkpatrick P. Belatacept. Nat Rev Drug Discov. 2011;10(9):655–6. doi:10.1038/nrd3536.
Wing K, Yamaguchi T, Sakaguchi S. Cell-autonomous and –non-autonomous roles of CTLA-4 in immune regulation. Trends Immunol. 2011;32(9):428–33. doi:10.1016/j.it.2011.06.002.
Ying H, Yang L, Qiao G, Li Z, Zhang L, Yin F, Xie D, Zhang J. CTLA-4-B7 interaction suppresses Th17 cell differentiation. J Immunol. 2010;185:1375–8.
Yokosuka T, Kobayashi W, Takamatsu M, Sakata-Sogawa K, Zeng H, Hashimoto-Tane A, Yagita H, Tokunaga M, Saito T. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. Immunity. 2010;33(3):326–39.

CTLA4-Ig

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Synonyms

Abatacept; Belatacept

Definition

CTLA4-Ig (abatacept) (Orencia™, Bristol-Myers Squibb) has been developed for the treatment of autoimmune disease and is approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of moderately to severely active adult rheumatoid arthritis (RA) and moderately to severely active juvenile idiopathic arthritis in children 6 years of age and older.

Introduction

Soluble CTLA4-Ig binds to CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells, blocking their interaction with CD28 on T cells and inhibiting T cell activation in vivo (Linsley et al. 1992). Two closely related CTLA4-Ig molecules have been developed for the treatment of autoimmune disease and prevention of graft rejection in kidney transplantation. Abatacept (Orencia™, Bristol-Myers Squibb) has been approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of moderately to severely active adult RA and moderately to severely active juvenile idiopathic arthritis in children 6 years of age and older. Abatacept consists of the extracellular domain of human CTLA4 linked to the hinge, CH2, and CH3 domains of human IgG_1. In its development, four amino acid mutations were introduced into the constant regions of the IgG portion of the molecule to improve protein production and abrogate the effects of antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity.

Belatacept (Nulojix™, Bristol-Myers Squibb), a daughter molecule of abatacept, is approved for the prevention of graft rejection in kidney transplantation. Belatacept is structurally identical to abatacept except for two amino acid substitutions in the extracellular ligand-binding domain (L104→E and A29→Y) that confer fourfold and twofold greater binding avidity to CD86 and CD80, respectively, compared with its parent compound.

CTLA4

The therapeutic mechanisms of CTLA4-Ig derive from the extracellular domain of the CTLA4 molecule, an inhibitory receptor expressed on activated but not resting $CD4^+$ and $CD8^+$ T cells. CTLA4 shares two ligands, CD80 and CD86, with CD28, a homologous co-stimulatory receptor constitutively expressed on naive T cells. CD28, the most widely studied co-stimulatory receptor on T cells, plays a major role in the induction and maintenance of T cell responses. It is the second signal of the 2-signal model of T cell activation. In this model, T cell activation depends on two signals: (1) the binding of the T cell receptor (TCR) to a major histocompatibility complex (MHC)-bound antigen on the antigen-presenting cell and (2) a co-stimulatory signal such as the binding of CD28 on the T cell to CD80 and CD86 on the antigen-presenting cell. TCR engagement by the MHC-antigen complex in the absence of a second signal leads to anergy of the T cell. Thus, depending on the strength of co-stimulation, the antigen-presenting cell may either trigger immunity (both signals) or peripheral tolerance (signal 1 alone). In general, naïve T cell responses are more dependent on a strong co-stimulatory signal than memory T cell responses.

The extracellular domain of CTLA4 has complex immunoregulatory effects. Once CTLA4 is upregulated on the surface of T cells shortly after cellular activation, it binds to CD80 and CD86, which results in a potent negative signal that downregulates the T cell response (Walunas et al. 1996). CTLA4 may also inhibit T cell activation by competing directly with CD28 for binding to CD80 and CD86. It has a competitive advantage over CD28 in this context because of its higher affinity for CD80 and CD86. CTLA4 can also generate a "reverse signal" through CD80 and CD86 and trigger the production of indoleamine 2, 3-dioxygenase (IDO) (Mellor and Munn 2004). IDO is an enzyme that catalyzes the degradation of tryptophan in many different types of cells, including dendritic cells. The restricted availability of tryptophan and the increased production of its metabolites, such as kynurenine, inhibit T cell proliferation and promote T cell apoptosis. CTLA4 is also likely to be important in the control of T regulatory cells. CTLA4 is constitutively expressed at high levels on the surface of $CD4^+FoxP3^+$ T regulatory cells. Upon antigen stimulation, its engagement by CD80 and CD86 may lead to the activation of $CD4^+FoxP3^+$ T regulatory cells and their acquisition of suppressive function (Rosenblum et al. 2011).

CTLA4-Ig

Studies in mice and nonhuman primates have shown that administration of CTLA4-Ig suppresses a mixed lymphocyte reaction, prevents allograft rejection, and inhibits T cell-dependent antibody responses (Linsley et al. 1992). In addition, CTLA4-Ig treatment has been shown to ameliorate disease in a variety of autoimmune models, including murine lupus, collagen-induced arthritis, experimental autoimmune encephalomyelitis, and diabetes. In these studies, therapy with CTLA4-Ig potently suppressed antigen-specific T cell proliferation, T-dependent B cell responses, and cytokine production by virtue of its ability to block the CD28:CD80/86 co-stimulatory pathway. However, joint inflammation was not affected when abatacept was given to a SCID mouse engrafted with human rheumatoid arthritis (RA) synovial tissue, implying this agent may not act directly on the inflamed synovium in RA, but rather at a distant site, such as secondary lymphoid tissue. CTLA4-Ig treatment has also been shown in these animal models to stimulate the production of IDO by antigen-presenting cells, resulting in suppression of T cell proliferation (Grohmann et al. 2003). Since CTLA4-Ig treatment has been reported to have both positive and negative effects in mice on the $CD4^+FoxP3^+$ T regulatory cell population, its overall impact on this regulatory subset is unclear and may vary according to disease state.

Less is known about the immunoregulatory effects of CTLA4-Ig therapy in humans. The ability of abatacept to interfere with T cell priming has been demonstrated in patients with psoriasis vulgaris. In this study, abatacept treatment decreased antibody responses to a T cell-dependent neoantigen, an inhibitory effect that was reversible upon stopping the drug (Abrams et al. 1999). In a subsequent study, a single infusion of abatacept 750 mg given at −2 weeks, +2 weeks, and +8 weeks following immunization was found to have no significant effects on the antibody response to tetanus toxoid (memory T cell-dependent antigen) or pneumococcal vaccine (memory T cell-independent antigen), although geometric mean titers of anti-tetanus and anti-pneumococcal titers tended to be lower in subjects vaccinated 2 weeks after receiving abatacept than the later time point (Tay et al. 2007).

As mentioned earlier, abatacept may induce IDO and catalyze tryptophan degradation in mice, leading to suppression of T cell activation. However, attempts to confirm these effects in humans have not produced consistent results. Human dendritic cells, in particular, have not been clearly and reproducibly shown in vitro and in vivo to increase IDO expression after CTLA4-Ig treatment. In kidney transplant recipients, belatacept therapy may have effects on the regulatory T cell population where it has been shown to increase the percentage of $CD4^+CD25^+FoxP3$ T regulatory cells infiltrating the kidneys undergoing acute cellular rejection (Bluestone et al. 2008; Furuzawa-Carballeda et al. 2010).

CTLA4-Ig as Immunotherapy: Rheumatoid Arthritis

Abatacept was initially developed as an intravenous preparation for the treatment of RA. To enhance dosing flexibility, abatacept may now also be given by the subcutaneous route, which has the same efficacy as the intravenous form. Several large, randomized, controlled trials have been performed evaluating the efficacy and safety of abatacept therapy for RA. In the AIM (Abatacept in Inadequate Responders to Methotrexate) study, for example, 652 patients with active RA despite methotrexate therapy were randomly allocated to receive treatment with abatacept ($n = 433$) or placebo ($n = 219$) while they continued their methotrexate therapy. At 1 year, the American College of Rheumatology (ACR)20 response rates in the abatacept and placebo groups were 73.1 % and 39.7 %, respectively ($P < 0.001$). An ACR response, a dichotomous outcome, corresponds to a 20 %

CTLA4-Ig, Table 1 Phase III clinical trials of abatacept therapy for adults with RA

Phase III study	N = number of subjects	Duration	Clinical efficacy endpoints	
			ACR 50 response	DAS28 remission (<2.6)
AGREE	509	1 year	Abatacept + MTX versus MTX + placebo (57.4 % vs. 42.3 %; $p < 0.001$)	Abatacept + MTX versus MTX + placebo (41.4 % vs. 23.3 %; $p < 0.001$)
AIM	652	1 year	Abatacept + MTX versus MTX + placebo (48.3 % for abatacept vs. 18.2 %; $p < 0.001$)	Abatacept + MTX versus MTX + placebo (23.8 % vs. 1.9 %; $p < 0.001$)
ATTAIN[a]	391	6 months	Abatacept versus placebo (20.3 % vs. 3.8 % ; $p < 0.001$)	Abatacept versus placebo (10.0 % vs. 0.8 %; $p > 0.001$)
ATTEST	431	6 months	Abatacept + MTX versus MTX + placebo (40.4 % vs. 20.0 %; $p < 0.001$)	Abatacept + MTX versus MTX + placebo (11.3 % vs. 2.9 %)

Abbreviations: *MTX* methotrexate, *AGREE* Abatacept trial to Gauge Remission and joint damage progression in methotrexate-naïve patients with Early Erosive rheumatoid arthritis (Westhovens et al. 2009), *AIM* Abatacept in Inadequate responders to Methotrexate (Kremer et al. 2006), *ATTAIN* Abatacept Trial in Treatment of Anti-TNF Inadequate responders (Genovese et al. 2005), *ATTEST* Abatacept or infliximab versus placebo, a Trial for Tolerability, Efficacy and Safety in Treating rheumatoid arthritis (Schiff et al. 2008). [a]Background oral disease-modifying antirheumatic drugs (DMARDS): methotrexate, azathioprine, penicillamine, gold, hydroxychloroquine, chloroquine, leflunomide, sulfasalazine.

or 50 % (i.e., ACR20, ACR50) improvement in a core set of 7 clinical and laboratory variables used to measure disease activity that includes the tender and swollen joint count, physician's assessment of disease activity, patient's assessment of disease activity, patient's assessment of pain, patient's assessment of physical function, and serum levels of an acute-phase reactant (C-reactive protein [CRP] or erythrocyte sedimentation rate [ESR]). Clinical remission, as defined by the Disease Activity Scale (DAS) 28, was observed in 23.8 % of the abatacept group but only 1.9 % of the placebo group (Kremer et al. 2006). The DAS 28 is a continuous score that also measures disease activity and is based on the number of tender and swollen joints (total of 28 joints), a patient's global health assessment, and serum level of acute-phase reactant (CRP or ESR). Patients taking the combination of abatacept and methotrexate showed about one-half the rate of radiographic progression of joint damage as those receiving placebo and methotrexate. Another randomized, placebo-controlled, clinical trial (AGREE) subsequently found that abatacept was also efficacious therapy for patients with early RA (Westhovens et al. 2009). Early RA was defined in this trial as disease of less than 2 years and included only those patients with a positive test for rheumatoid factor and/or anti-CCP antibodies or joint erosions on their radiographs. In this study, patients with limited or no previous exposure to methotrexate therapy (the usual first-line treatment for RA) were randomly allocated to receive treatment with abatacept and methotrexate or methotrexate alone. The patients receiving the combination of abatacept and methotrexate achieved a DAS28 remission in 41.4 % of cases as opposed to 23.3 % of patients receiving methotrexate alone ($p < 0.001$). Compared to the methotrexate-only group, the abatacept plus methotrexate combination also showed less radiographic progression of disease. Such combination therapy may be an option for patients with early RA and poor prognostic factors.

Three-year data from the AIM trial also provided valuable information about the long-term safety of abatacept therapy for RA (Kremer et al. 2011). By the third year of the AIM trial, 359 (83 %) of the original 433 patients receiving abatacept and 175 (80 %) of the 219 receiving placebo had remained in the study and were available for assessment. The most common adverse

event was infection with an incidence rate of 70.8 per 100 patient-years; the incidence rate for serious infection was 3.2 per 100 patient-years. Ten deaths occurred in the cumulative period and were primarily related to infection. The overall incidence ratio for malignancy was comparable with the rate of malignancy in patients with RA that are treated with conventional therapy. Other safety studies have shown that infection rates are higher when abatacept is combined with a biologic DMARD (e.g., etanercept, adalimumab, infliximab) as opposed to a traditional DMARD such as methotrexate. Therefore, abatacept is not combined with another biologic DMARD in routine clinical practice.

Abatacept is also effective for the treatment of juvenile idiopathic arthritis (JIA). For example, in a double-blind randomized controlled withdrawal trial, abatacept was found to be a safe and clinically effective therapy for JIA that produced tangible life benefits to children with this disease (Ruperto et al. 2008).

CTLA4-Ig Therapy for Type I Diabetes

Type I diabetes (DM1) results from the destruction of the insulin-producing islet cells of the pancreas by an immune-mediated, chronic inflammatory process. Although DM1 may be managed by insulin replacement therapy, its course may be associated with both short- and long-term complications. Therapies aimed at arresting islet cell destruction, such as those targeting CD3 and CD20, have seen only limited success thus far in clinical trials. Since T cells are believed to be central to the pathogenesis of DM1, abatacept has also been investigated as a possible treatment for this autoimmune disease. To this end, a phase II randomized, placebo-controlled trial of abatacept has been recently completed in patients with recent-onset type 1 diabetes showing abatacept therapy to be associated with a significantly higher geometric area under the curve (AUC) of the stimulated C-peptide level at 2 years compared with the placebo group (Orban et al. 2011). The serum level of C-peptide reflects the amount of residual insulin secretion by the pancreas. Treatment with abatacept delayed the reduction in stimulated C-peptide levels by 9.6 months compared with the placebo group; however, the initial benefit of the abatacept infusion was observed mainly in the first 6 months as the subsequent decline in stimulated serum C-peptide levels paralleled that of the placebo group. These observations are consistent with a "window of opportunity" during the early stages of DM1 when the chronic inflammatory response in the pancreatic islets may be inhibited to reverse the inexorable decline in β-cell function.

CTLA4-Ig for Transplantation

In nonhuman primate transplant models, abatacept was found to be relatively ineffective for preventing the rejection of a transplanted organ because it did not completely block co-stimulation mediated by CD80 and CD86. To overcome this shortcoming, Bristol-Meyers Squibb developed belatacept from the parent abatacept molecule through a mutagenesis and screening strategy that conferred it with more potent immunosuppressive properties. Belatacept produces a tenfold greater inhibition of T cell activation in vitro than abatacept.

Unlike abatacept, belatacept was effective in a nonhuman primate model where in combination with standard immunosuppressive therapy prolonged the survival of renal allografts compared to a standard cyclosporine-based regimen. Because it was effective in primates, the drug was then studied further in human trials. The Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT), a phase III randomized, controlled trial, tested the efficacy and safety of belatacept for the prevention of graft rejection in 527 patients who had received a living donor or deceased donor kidney (Vincenti et al. 2010). All of the patients received induction therapy with basiliximab and maintenance therapy with mycophenolate mofetil and corticosteroids. Patients were randomized into three groups: more intensive (MI) belatacept, less intensive (LI)

belatacept, and cyclosporine (CsA). The co-primary endpoints were patient/graft survival, composite renal impairment, and incidence of acute rejection at 12 month. The patients receiving the MI and LI regimens had 95 % and 97 % patient/graft survival, respectively, compared with 93 % graft survival in the patients treated with CsA. The composite renal impairment endpoint was also significantly better in both of the belatacept treatment groups compared to the cyclosporine-treated patients. While belatacept therapy was associated with a higher rate of acute rejection than CsA therapy (MI 22 %; LI: 17: CsA 7 %), it led to less chronic allograft nephropathy (MI: 18 %; LI: 24 %; CsA: 32 %).

The other large phase III study was termed BENEFIT-EXT for extended criteria donors and included patients that had received a kidney transplant from a donor defined as greater than 60 years old or greater than 50 years old with at least two risk factors such as stroke, hypertension, or serum creatinine greater than 1.5 mg/dL (Durrbach et al. 2010). The study design was otherwise the same as that of the BENEFIT trial. Both belatacept groups were non-inferior to cyclosporine based on the co-primary endpoint at 12 months of patient/graft survival and superior to CsA with respect to the co-primary endpoint of composite renal impairment. In contrast to the results from BENEFIT, this study did not find any significant differences between the three treatment groups in the incidence of acute rejection (MI: 18 %; LI: 18 %; CsA 14 %).

Based on these two phase III studies, it appears that substituting belatacept for a calcineurin inhibitor for the prevention of kidney allograft rejection may lead to an improved glomerular filtration rate, lower blood pressure, and improved lipid profile. The finding of a greater incidence of acute rejection in patients treated with belatacept as opposed to CsA in BENEFIT raises important questions about the efficacy of co-stimulatory blockade in this situation. However, the cellular rejections in the belatacept groups tended to be milder than the CsA group, allaying some of this concern. A possible explanation for these milder cellular rejections may relate to the belatacept-related expansion of $FoxP3^+$ T regulatory cells in the grafts. It has also been recognized that belatacept may promote the development of posttransplant lymphoproliferative disease (PTLD). The risk for this complication may be minimized by excluding patients without serologic evidence of prior Epstein-Barr virus infection and avoiding lymphocyte-depleting agents in the setting of acute rejection. Belatacept is currently approved for the prophylaxis of organ rejection in adult patients who are seropositive for previous Epstein-Barr virus infection receiving a kidney transplant in combination with basiliximab, mycophenolate mofetil, and corticosteroids. Its use has not been established for the prevention of rejection in patients with other transplanted organs or tissues.

Conclusion

In summary, CTLA4-Ig represents a novel approach to immunosuppressive therapy by targeting a specific co-stimulatory pathway involved in T cell activation. Abatacept is approved for the treatment of RA and JIA, providing direct evidence for an important role of T cells in the pathogenesis of these diseases. Much more will be learned from further studies investigating its clinical efficacy and safety in other settings, such as type 1 diabetes, lupus, and psoriatic arthritis. In transplant medicine, belatacept is a promising alternative to CsA for prevention of kidney allograft rejection. Future studies inhibiting this pathway in humans will be necessary to improve our understanding of the mechanisms of CTLA4-Ig in vivo and its relevance for fine-tuning the balance between immunity (autoimmunity) and regulation for therapeutic gain.

Cross-References

- ▶ B7 and CD28 Families
- ▶ CTLA-4
- ▶ Cytotoxic T Lymphocytes
- ▶ Juvenile Diseases: SLE in Children
- ▶ Juvenile Idiopathic Arthritis
- ▶ Juvenile Idiopathic Arthritis: Pathogenesis, Presentation, and Treatment

- ▶ Psoriasis
- ▶ Rheumatoid Arthritis, Biologics in its Treatment
- ▶ Rheumatoid Arthritis, Clinical Features
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver

References

Abrams JR, Lebwohl MG, et al. CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. J Clin Invest. 1999;103(9):1243–52.

Bluestone JA, Liu W, et al. The effect of costimulatory and interleukin 2 receptor blockade on regulatory T cells in renal transplantation. Am J Transplant. 2008;8(10):2086–96.

Durrbach A, Pestana JM, et al. A phase III study of belatacept versus cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study). Am J Transplant. 2010;10(3):547–57.

Furuzawa-Carballeda J, Lima G, et al. High levels of IDO-expressing CD16+ peripheral cells, and Tregs in graft biopsies from kidney transplant recipients under belatacept treatment. Transplant Proc. 2010;42(9):3489–96.

Genovese MC, Becker JC, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. N Engl J Med. 2005;353(11):1114–23.

Grohmann U, Fallarino F, et al. Tolerance, DCs, and tryptophan: much ado about IDO. Trends Immunol 2003;24(5):242–248.

Kremer JM, Genant HK, et al. Effects of abatacept in patients with methotrexate-resistant active rheumatoid arthritis: a randomized trial. Ann Intern Med. 2006;144(12):865–76.

Kremer JM, Russell AS, et al. Long-term safety, efficacy and inhibition of radiographic progression with abatacept treatment in patients with rheumatoid arthritis and an inadequate response to methotrexate: 3-year results from the AIM trial. Ann Rheum Dis. 2011;70(10):1826–30.

Linsley PS, Greene JL, et al. Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes. J Exp Med. 1992;176(6):1595–604.

Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol. 2004;4(10):762–74.

Orban T, Bundy B, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. Lancet. 2011;378(9789):412–9.

Rosenblum MD, Gratz IK, et al. Response to self antigen imprints regulatory memory in tissues. Nature. 2011;480(7378):538–42.

Ruperto N, Lovell DJ, et al. Abatacept in children with juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled withdrawal trial. Lancet. 2008;372(9636):383–91.

Schiff M, Keiserman M, et al. Efficacy and safety of abatacept or infliximab vs placebo in ATTEST: a phase III, multi-centre, randomised, double-blind, placebo-controlled study in patients with rheumatoid arthritis and an inadequate response to methotrexate. Ann Rheum Dis. 2008;67(8):1096–103.

Tay L, Leon F, et al. Vaccination response to tetanus toxoid and 23-valent pneumococcal vaccines following administration of a single dose of abatacept: a randomized, open-label, parallel group study in healthy subjects. Arthritis Res Ther. 2007;9(2):R38.

Vincenti F, Charpentier B, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). Am J Transplant. 2010;10(3):535–46.

Walunas TL, Bakker CY, et al. CTLA-4 ligation blocks CD28-dependent T cell activation. J Exp Med. 1996;183(6):2541–50.

Westhovens R, Robles M, et al. Clinical efficacy and safety of abatacept in methotrexate-naive patients with early rheumatoid arthritis and poor prognostic factors. Ann Rheum Dis. 2009;68(12):1870–7.

Cutaneous Vasculitis

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Synonyms

Cutaneous manifestations of vasculitis

Definition

The vasculitides or vasculitis is characterized by inflammation of and reactive damage to blood vessel walls.

Cutaneous Vasculitis

Classification of the vasculitides has been a challenging process. Traditionally, the vasculitides have been categorized based on the dominant size or type of the vessels most commonly affected by a given disease. The initial classification scheme suggested by Zeek in 1952 did not include several forms of vasculitis. The most widely accepted current classification schemes, including the American College of Rheumatology (ACR) classification criteria introduced in 1990 and the subsequent revisions in the Chapel Hill Consensus Conference (CHCC) in 1994, do not address all forms of primary vasculitis. Also, small-vessel disease and skin involvement may occur in many of the proposed groups, making the schemes less practical. The classification of vasculitis syndromes continues to evolve. The association with antineutrophil cytoplasmic antibodies (ANCA) and the concept of ANCA-associated vasculitis (AAV) have been recently added to proposed classification schemes including a new consensus algorithm.

Cutaneous vasculitis is a broad clinical entity. Many forms of vasculitis can affect the skin because of the large number of vessels in the dermis and subcutaneous tissue. Cutaneous vasculitis may be a primary process or may occur secondary to other disease processes such as infection, noninfectious inflammation due to a drug or systemic autoimmune disease, and malignancy. Infections and drug reactions are the most common etiologies of cutaneous vasculitis and account for 23 and 20 % of cases, respectively. Hepatitis C virus, hepatitis B virus, HIV, cytomegalovirus, parvovirus B19, varicella zoster virus, streptococcus, Staphylococcus aureus, neisseria, and rickettsia are the most common infectious causes of cutaneous vasculitis. Penicillins, cephalosporins, sulfonamides, thiazides, allopurinol, phenytoin, tumor necrosis factor (TNF) inhibitors, rituximab, and cocaine are among the most common triggers of drug-induced cutaneous vasculitis. Type II antibody-mediated and type III immune-complex-mediated hypersensitivity are the most common pathogenic mechanisms of cutaneous vasculitis.

The caliber of the vessels primarily involved determines the clinical morphology of cutaneous lesions. For example, small-vessel disease (capillaries, postcapillary venules, and nonmuscular arterioles <50 μm in the superficial and mid-dermis) causes palpable purpura, urticarial lesions, and superficial ulcers. Cutaneous small-vessel vasculitis (CSVV), urticarial vasculitis, cryoglobulinemic vasculitis, and Henoch-Schonlein purpura (HSP) are the most common small-vessel vasculitides. Medium-sized disease (50–150 μm vessels with muscular walls in the deep dermis and subcutis) causes subcutaneous nodules, nodular and deep ulcers, livedo reticularis (presenting as a red-blue, reticulated vascular network) and livedo racemosa (presenting as an irregular, branched, vascular pattern), purpura, and necrosis. The most common medium-vessel vasculitis is polyarteritis nodosa (PAN).

Other cutaneous lesions such as petechiae, patches, necrotic plaques, bullous lesions, and splinter hemorrhages may also be observed. Some vasculitides including microscopic polyangiitis, granulomatosis with polyangiitis (Wegener's) (GPA), and Churg-Strauss syndrome (CSS) can involve both small- and medium-sized vessels. The focus of this entry is small-vessel vasculitis, cutaneous manifestations of other types of vasculitides (e.g., PAN), and other systemic autoimmune disease or neoplasms that can manifest as cutaneous vasculitis (Table 1 and 2).

An appropriate diagnostic evaluation can guide laboratory work-up and therapy in cutaneous vasculitis. The initial approach should include a thorough history and physical examination focusing on new medications, symptoms of infection, and timing of the development of vasculitis. All patients should be questioned regarding symptoms that suggest systemic involvement such as fever, chills, fatigue, night sweats, weight loss, abdominal pain, melena, arthralgia, myalgia, muscle weakness, discoloration of the urine, hemoptysis, cough, dyspnea, wheezing, and paresthesias. Symptoms of viral hepatitis and systemic autoimmune disease should also be assessed.

Cutaneous Vasculitis, Table 1 Cutaneous manifestations in different types of cutaneous vasculitis

Types of cutaneous vasculitis	Cutaneous manifestations
Small-vessel vasculitides	
Cutaneous small-vessel vasculitis	Most common: palpable purpura, urticarial papules
	Less common: petechiae, vesiculobullous lesions, shallow ulcers, mucosal shallow ulcers
Cryoglobulinemic vasculitis	Most common: palpable purpura
	Less common: ecchymoses, papules, nodules, hemorrhagic crusts, ulcers, livedo reticularis, livedo racemosa, urticarial papules, infarcts, mucosal lesions
Urticarial vasculitis	Most common: urticarial papules, palpable purpura
	Less common: petechiae, livedo reticularis, erythema-multiforme, bullous lesions
Henoch-Schonlein purpura	Most common: palpable purpura, urticarial papules
	Less common: petechiae, vesiculobullous lesions, shallow ulcers, ecchymoses, macules, papules
Erythema elevatum diutinum	Most common: papules, nodules, plaques
	Less common: bullous lesions, ulcers, hemorrhagic lesions
Medium-vessel vasculitis	
Polyarteritis nodosa	Most common: nodules, deep ulcers, livedo **reticularis**
	Less common: bullous eruptions, palpable purpura, infarction and gangrene of the fingers and toes, livedo racemosa, large ulcers
Mixed (small and medium)-vessel vasculitides	
Granulomatosis with polyangiitis (Wegener's)	Most common: palpable purpura, deep ulcers with corrugated borders, nodules
	Less common: papulonecrotic lesions, oropharyngeal ulcers, livedo racemosa, urticaria, livedo reticularis, vesicles, infarcts, splinter hemorrhages
Churg-Strauss syndrome	Most common: palpable purpura, nodules
	Less common: ulcers, macules, papules, petechiae, ecchymoses, papulonecrotic lesions, urticaria, livedo racemosa, infarcts, livedo reticularis

Skin biopsy is an important part of the evaluation of all suspected cases of cutaneous vasculitis. Also, direct immunofluorescence (DIF) should generally be performed to identify immunoglobulin and complement deposits within the skin, particularly to determine if IgA is the predominant immunoglobulin, as seen with HSP. The DIF biopsies should be done on early lesions, preferably before 24 h. The ideal time for skin biopsy for routine histology is between 24 and 48 h after the development of the lesion. Prior to 24 h, fibrinoid necrosis may not yet be present and beyond 48 h, the inflammatory infiltrate begins to be replaced by macrophages and lymphocytes.

The type of skin biopsy affects the size of vessels available for pathological evaluation. Punch biopsies at least 4 mm in diameter are recommended for evaluation of skin lesions suggestive of involvement of vessels in the superficial to mid-dermis (palpable purpura and urticarial lesions). Deeper punch biopsies are recommended for the evaluation of skin lesions suggestive of involvement of vessels in the mid-dermis to subcutaneous tissue (subcutaneous nodules, nodular and deep ulcers, and livedo racemosa). Shave biopsies are not recommended since they are not helpful in the evaluation of the vessels in the mid- and deep dermis.

The location of biopsy is also important. Biopsies can be taken from any site of palpable purpura or petechiae. Biopsies of livedo racemosa should be taken from the pale center of an erythematous periphery. In ulcerations, biopsy should be performed from the edge of the ulcer. Biopsies of livedo reticularis are not recommended since they often show nonspecific histopathologic findings. The initial laboratory work-up should include complete blood count with differential, blood urea nitrogen (BUN), creatinine, liver function tests, and urinalysis

Cutaneous Vasculitis, Table 2 Diagnostic pearls and differential diagnosis in different types of cutaneous vasculitis

Types of cutaneous vasculitis	Diagnostic pearls	Differential diagnosis
Small-vessel vasculitides		• **Macular purpura (trauma, chronic sun exposure, glucocorticoid therapy, anticoagulant therapy)** • Noninfectious **inflammatory disorders** (drug eruptions, stasis dermatitis) • **Pigmented purpuric dermatoses** • **Arthropod bites** • **Meningococcemia** • Rickettsial and viral infections (postherpetic eruptions) • **Platelet deficiencies or dysfunction** • Ascorbic acid (vitamin C) deficiency • **Cholesterol or septic emboli** • **Hypercoagulable and thrombotic disorders** (antiphospholipid syndrome) • Amyloidosis • Atrophie blanche • Purpura fulminans • Strongyloidiasis • Cutaneous vasculitis secondary to other systemic autoimmune disease • Sarcoidosis • Superficial thrombophlebitis • Inflammatory bowel disease • Cutaneous lymphomas • Lichenoid dermatoses
Cutaneous small-vessel vasculitis	• No systemic disease (may present with fatigue or mild constitutional symptoms) • Raised, nonblanchable purpuric lesions affecting the dependent sites, such as lower extremities and back • IgM, IgA, IgG, C3 in the vessel wall on skin biopsy	
Cryoglobulinemic vasculitis	• Constitutional symptoms • Raised, nonblanchable purpuric lesions affecting the dependent sites, such as lower extremities and back • Polyneuropathy (axonal sensorimotor) • Renal disease • Presence of cryoglobulins • HCV antibody or HCV RNA-positive • IgM in the vessel wall on skin biopsy	
Urticarial vasculitis	• Constitutional symptoms • Painful urticarial papules persisting more than 24 h • Angioedema/abdominal pain • Raynaud's phenomenon • Arthralgias/arthritis • Renal disease • COPD/asthma • Hypocomplementemia (in hypocomplementemic urticarial vasculitis subtype) • C3, IgG, fibrin in the vessel wall on skin biopsy	
Henoch-Schonlein purpura	• Constitutional symptoms • Raised, nonblanchable purpuric lesions affecting the dependent sites, such as lower extremities and back • Abdominal pain • Arthralgias/arthritis • Renal disease • IgA in the vessel wall on skin biopsy	
Erythema elevatum diutinum	• No systemic involvement • Symmetrical, violaceous, red, or brown, firm, smooth papules, nodules, or plaques over the extensor surfaces of the extremities, particularly joints. • The lesions tend to increase in the number and size with time • Peripheral keratitis	
Medium-vessel vasculitis		• Infective endocarditis • Mycotic aneurysm • Hepatitis B or C infection • HIV • **Hypercoagulable and thrombotic disorders** (antiphospholipid syndrome) • **Cholesterol or septic emboli** • Small-vessel vasculitides • Granulomatosis with polyangiitis (Wegener's)
Polyarteritis nodosa	• Constitutional symptoms • Tender painful subcutaneous nodules, deep ulcers, and livedo **reticularis** • Peripheral neuropathy (mononeuritis multiplex) • ANCA negative	

(*continued*)

Cutaneous Vasculitis, Table 2 (continued)

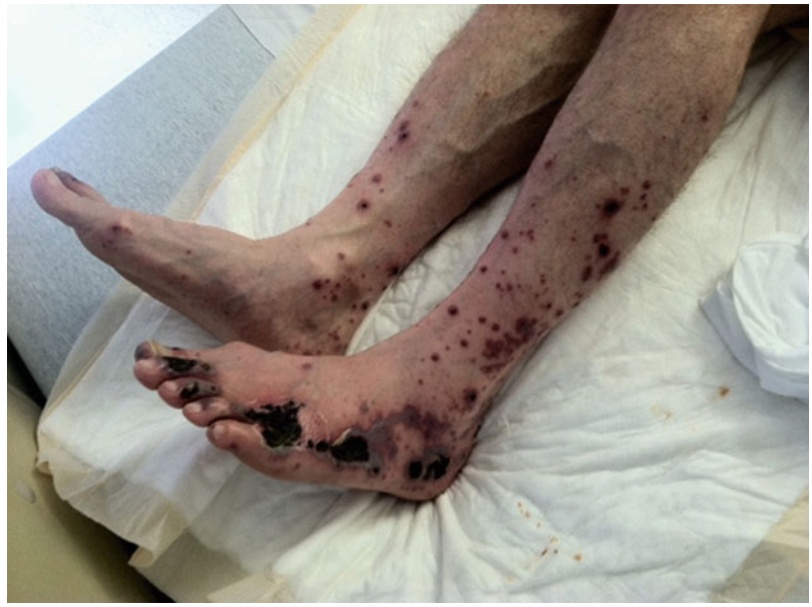
Types of cutaneous vasculitis	Diagnostic pearls	Differential diagnosis
Mixed (small and medium)-vessel vasculitides		• Polyarteritis nodosa • Churg-Strauss syndrome • Microscopic polyangiitis • Anti-glomerular basement membrane disease • Small-vessel vasculitides • Chronic eosinophilic pneumonia • Aspirin-exacerbated respiratory disease • Allergic bronchopulmonary aspergillosis
Granulomatosis with polyangiitis (Wegener's)	• Constitutional symptoms • Raised, nonblanchable purpuric lesions affecting the dependent sites, such as lower extremities and back; deep ulcers with corrugated borders, nodules • Presence of circulating PR3-ANCA (C-ANCA) • Granulomatous inflammation on nasal biopsy • Pauci-immune necrotizing glomerulonephritis	
Churg-Strauss syndrome	• Constitutional symptoms • Raised, nonblanchable purpuric lesions affecting the dependent sites, such as lower extremities and back; nodules • Presence of asthma/rhinosinusitis/allergies • Eosinophilic pneumonia/eosinophilic gastroenteritis • Peripheral neuropathy (mononeuritis multiplex) • Clinical signs of heart failure and cardiac rhythm abnormalities • Presence of prominent eosinophilia ($\geq$ 1,500 cells/microL and/or $>$ 10 % eosinophils on differential leukocyte count) • Presence of circulating p-ANCA with anti-MPO specificity	

(including thorough examination of the urinary sediment for cellular casts). When the etiology of biopsy-proven cutaneous vasculitis is unclear, other relevant testing can be performed such as hepatitis B and C serologies, complement levels (CH50, C3, and C4), ANCA, cryoglobulins, HIV, rheumatoid factor, ANA, anti-dsDNA, anti-Smith, anti-Ro, anti-La, and anti-RNP antibodies. Chest x-ray is recommended in patients with pulmonary symptoms (Hunder et al. 1990; Jennette et al. 1994; Jennette and Falk 1997; Stone and Nousari 2001; Fiorentino 2003; Carlson and Chen 2006; Grzeszkiewicz and Fiorentino 2006; Carlson et al. 2006; Xu et al. 2009; Carlson 2010).

Small-Vessel Vasculitides

Type III immune-complex-mediated hypersensitivity plays an important role in small-vessel vasculitides since C5b-9, the terminal components of the complement cascade, are detected in most of these conditions (Black 1999; Ramos-Casals et al. 2000).

Common cutaneous manifestations of small-vessel vasculitis include palpable purpura and urticarial lesions (Fig. 1). The skin lesions primarily affect the dependent sites, such as lower extremities and back, due to increased hydrostatic pressure. Palpable purpura results from extravasation of red blood cells from the vessel lumen into the dermis and superficial perivascular neutrophilic infiltrates. Therefore, they do not blanch upon pressure. Palpable purpura can be asymptomatic, pruritic, or with a burning pain and resolves with residual postinflammatory hyperpigmentation. Urticarial lesions are thought to result from a type III immune-complex-mediated hypersensitivity reaction. Urticarial lesions due to vasculitis last longer than 24 h. They may be associated with



Cutaneous Vasculitis, Fig. 1 Palpable purpura is the most common cutaneous manifestation of small-vessel vasculitis. It can be associated with shallow ulcers

a burning sensation. The urticarial lesions of vasculitis typically resolve with postinflammatory hyperpigmentation (Hautmann et al. 1999).

The major histopathologic features of small-vessel vasculitis include the presence of inflammatory infiltrates in and/or around blood vessel walls, disruption and/or destruction of vessel walls by the inflammatory infiltrate, fibrin deposition within the vessel wall or lumen (fibrinoid necrosis).

Cutaneous Small-Vessel Vasculitis (CSVV) (Previously Termed Hypersensitivity Vasculitis)

The term "hypersensitivity vasculitis" (HV), first introduced by Zeek in 1950, is a confusing term that has been used to include several types of small-vessel vasculitis, including those triggered by a known antigen such as a drug. The ACR classification system recognizes HV; however, their criteria do not distinguish between HV and other small-vessel vasculitis such as HSP which could be of prognostic value. The term "cutaneous leukocytoclastic angiitis" was then suggested in the CHCC classification criteria. This term has its limitation, however, with the term angiitis inappropriately broad and the term "leukocytoclastic" a histopathologic term indicating the presence of polymorphonuclear leukocytes (neutrophils) and fibrinoid necrosis within and around the vessel wall. Therefore, the term "cutaneous leukocytoclastic vasculitis" most appropriately refers to neutrophilic small-vessel vasculitis.

Cutaneous small-vessel vasculitis (CSVV) is a better term that has been used to refer to vasculitis involving the small blood vessels in the skin including neutrophilic, lymphocytic, and granulomatous vasculitides. By definition, patients with CSVV have isolated skin involvement and no systemic disease. An alternative term that has been used is "cutaneous necrotizing venulitis."

CSVV primarily involves the postcapillary venules in the dermis and usually presents with palpable purpura. A single crop of skin lesions can erupt simultaneously that proceeds through morbilliform stage and evolve to palpable purpura over few hours. In severe cases, CSVV may impact the deeper dermal postcapillary venules and therefore be associated with vesiculobullous lesions and shallow ulcers as well. Mucosal surfaces can be affected. A precipitating factor such as an infectious agent or a new medication may be identified in patients with CSVV. The diagnosis is one of exclusion. The majority of

patients with CSVV have a benign course, and the lesions usually resolve within several weeks. Recurrent disease can occur in approximately 10 % of patients. Cryoglobulinemia, arthralgia, and the absence of fever have been shown to be risk factors for recurrence.

The management of CSVV involves a work-up for potential underlying systemic disorders or an infection that could guide therapy. Conservative management including simple measures such as avoiding cold temperatures and sunlight, leg elevation, and reduction of activity is often successful. Antihistamines and nonsteroidal anti-inflammatory drugs (NSAIDs) have been used to alleviate burning and itching. Topical therapy with corticosteroid and antibiotic creams may be helpful in some patients, although there is no data to support their use. For refractory, relapsing, or extensive cases, escalating therapy may be required. Colchicine has been used for cutaneous and joint symptoms. Dapsone has also been used to alleviate cutaneous and joint manifestations. The use of colchicine or dapsone is based on anecdotal studies or small case series. Colchicine was not associated with any significant therapeutic effect in the only randomized controlled trial to date (Sais et al. 1995). Some experts recommend colchicine combined with dapsone, while others believe that colchicine should be used as a first-line and dapsone as a second-line therapy. Brief periods of high-dose oral corticosteroids or other immunosuppressive strategies may be warranted for severe systemic vasculitis. The immunosuppressive agents usually considered include methotrexate, azathioprine, mycophenolate mofetil, cyclosporine, and cyclophosphamide. Rituximab and TNF inhibitors have shown some efficacy in recent case reports (Stone and Nousari 2001; Fiorentino 2003; Carlson and Chen 2006; Grzeszkiewicz and Fiorentino 2006; Carlson et al. 2006; Xu et al. 2009; Sais et al. 1995).

Cryoglobulinemic Vasculitis (CV)

Cryoglobulinemic vasculitis (CV) is characterized by the presence of cryoglobulins and vessel wall injury. Cryoglobulins are a mix of serum immunoglobulins and complement that reversibly precipitate in the cold (under body temperature; 37 °C). Preliminary classification criteria have been proposed for CV (De Vita et al. 2011). These require validation. Cryoglobulins have been divided into three subtypes. Type I is composed of monoclonal IgM that is associated with plasma cell proliferative disorders, myeloma or Waldenstrom's macroglobulinemia. Type I usually causes a hypercoagulable state and vessel obstruction that leads to ischemic vasculopathy. Type II consists of monoclonal IgM directed against polyclonal IgG. Type III is polyclonal IgM directed against polyclonal IgG. Type II and III are termed mixed cryoglobulinemia due to the presence of more than one immunoglobulin isotype. Mixed cryoglobulinemia is most often due to hepatitis C virus infection. Other etiologies include HIV, connective tissue diseases, and lymphoproliferative disorders. Mixed cryoglobulinemia can be associated with deposition of IgM-IgG complexes in the walls of small vessels and subsequent complement activation, which results in inflammation of the vessel walls and vasculitis in 15 % of patients with circulating cryoglobulins.

The most common cutaneous manifestation and presenting feature in CV is palpable purpura, usually confined to the lower extremities. Ecchymoses, papules, and dermal nodules can also occur. Other cutaneous manifestations such as hemorrhagic crusts, ulcers, livedo reticularis, and mucosal lesions more commonly occur in type I cryoglobulinemia. Cutaneous manifestations are more common in antibody-positive HCV CV. Other major clinical manifestations of CV include arthralgia, lymphadenopathy, axonal sensorimotor polyneuropathy, hepatosplenomegaly, and renal disease. The diagnosis is made based on the history, presence of palpable purpura, hypocomplementemia, and high titers of circulating cryoglobulins. Biopsy of palpable purpuric lesions most often shows leukocytoclastic vasculitis. Direct immunofluorescence microscopy of acute lesions can detect IgM, IgG, and C3 complement deposits. HCV infection should be investigated in all patients diagnosed with CV by testing the serum or cryoprecipitate for HCV antibody or HCV RNA.

Patients with HCV-related CV should be treated for the underlying viral infection. Currently, interferon alpha, preferably pegylated, plus ribavirin should be considered for patients with normal renal function. Interferon alpha has been reported to be efficacious for HCV-negative CV as well, possibly due to its immunomodulatory effects. Combination therapy with rituximab has also been suggested to target cryoglobulin-producing B cells. In patients presenting with acute and severe disease, plasmapheresis in conjunction with corticosteroids and cyclophosphamide are indicated. The titer of cryoglobulins and complement levels do not correlate with severity of disease and cannot be used to monitor response to therapy (Ramos-Casals et al. 2000; De Vita et al. 2011).

Urticarial Vasculitis (UV)

UV is an entity consisting of urticarial eruption and histopathological evidence of leukocytoclastic vasculitis. It most commonly affects the postcapillary venules. UV is divided into normocomplementemic and hypocomplementemic (HUV) subtypes for prognostic purposes. Normocomplementemic UV is associated mild disease and is usually restricted to cutaneous sites. It is usually idiopathic but frequent associations include systemic autoimmune diseases. It can also occur in association with drugs, infections, hematologic malignancies, or exposure to UV light. UV is thought to result from the depositions of antigen-antibody complexes in the blood vessel walls which can activate the classical complement pathway, generating C3a and C5a. This causes mast cell degranulation and urticaria. The eliciting antigen is still largely unknown. In some patients, and particularly in hypocomplementemic UV, C1q autoantibodies may be present. UV is a rare disease but its exact incidence is uncertain. Approximately 5–10 % of patients with chronic urticaria have UV.

The lesions of UV can occur anywhere in the body. In contrast to simple urticaria, the UV wheals are painful with a burning quality and non-pruritic. Also the urticarial lesions in UV persist more than 24 h. The UV eruptions evolve into purpura, bruising, and postinflammatory hyperpigmentation. Other cutaneous manifestations of UV include livedo reticularis, erythema multiforme, or bullous lesions. Common systemic manifestations and associations include arthralgias, arthritis, renal disease, COPD, and asthma.

The initial approach to the patient with UV-like lesions should include a thorough history and physical examination, addressing prior and current urticarial lesions, preceding exposure to drugs or infections, and systemic symptoms. UV is considered a clinicohistopathologic entity, and a skin biopsy should always be performed. The histologic features include fragmentation of leukocytes with nuclear debris (leukocytoclasis), and fibrin deposition in and around the vessels. Immunofluorescence reveals deposits of immunoglobulins, complement, or fibrin around blood vessels.

Therapies for UV are based on small case series. Antihistamines are used for symptomatic control of pruritus. Dapsone, colchicine, pentoxifylline, hydroxychloroquine, and mycophenolate mofetil can be quite effective. Additional medications such as systemic glucocorticoids are frequently required for control of the illness and prevention of further urticarial lesions. Rituximab have also been used to treat the cutaneous disease. The management of systemic symptoms depends on the severity of the disease; referral to a physician with extensive experience in the management of vasculitides is usually recommended (Black 1999; Venzor et al. 2002).

Henoch-Schonlein Purpura (HSP)

HSP is clinically characterized by palpable purpura, arthralgia/arthritis, gastrointestinal symptoms, and renal disease. It primarily involves the postcapillary venules. HSP is primarily a childhood disease with a reported male-to-female ratio of approximately 1.5:1. HSP is thought to result from IgA deposition in the vessel walls; however, the triggering antigen and pathogenesis remain unknown. An upper respiratory tract infection usually precedes the disease. Palpable purpura is the hallmark cutaneous

manifestation of HSP and is present in almost all patients; however, the clinical manifestations may occur in any order. It typically begins with erythematous, macular, papular, or urticarial lesions which then coalesce and evolve into the symmetrically distributed crops of petechiae, palpable purpura, and ecchymoses. Occasionally, the skin can blister and ulcerate. The rash primarily affects the dependent sites, such as lower extremities and buttocks. Spread of palpable purpura above the waist has been shown to be a risk factor for renal disease.

The diagnosis is typically made clinically but can be confirmed by a skin biopsy, particularly in adult patients. Immunofluorescence microscopy often reveals IgA deposition in and around the vessel walls. Patients with HSP do not have thrombocytopenia or coagulopathy. Urinalysis should be obtained in all patients with HSP as hematuria is a typical feature; cellular casts are less common.

HSP is usually self-limited; however, in adults, it can be associated with significant renal disease and a worse prognosis compared with children. Treatment is largely supportive. NSAIDs and corticosteroids can be used for the relief of arthralgia/arthritis and gastrointestinal symptoms. IVIG, plasmapheresis, and a variety of cytotoxic agents have been used as the treatment for severe renal disease (Saulsbury 2010), but there is currently no consensus regarding indications for more aggressive treatment.

Erythema Elevatum Diutinum (EED)

EED is a rare form of small-vessel vasculitis that is limited to the skin. EED mostly affects the older adults, and there is a slight female predominance. Its cause is unknown. Though its pathogenesis is also uncertain, EED is thought to result from immune complex deposition in blood vessel walls. The onset of clinical symptoms in EED has been associated with infections (such as streptococcus and HIV), hematologic malignancies, IgA monoclonal gammopathy, connective tissue disorders, celiac sprue, and drugs. EED presents with symmetrical, violaceous, red, or brown, firm, smooth papules, nodules, or plaques over the extensor surfaces of the extremities, particularly joints. Lesions may also involve the buttocks, palms, and soles. Bullous lesions, ulcers, and hemorrhagic lesions are rare. The skin lesions in EED are mostly asymptomatic but can be painful or pruritic. The lesions tend to increase in the number and size with time. Ocular complications such as peripheral keratitis have been reported. Biopsy of early lesions typically shows leukocytoclastic vasculitis with prominent interstitial neutrophils. Biopsy of well-formed and chronic lesions may reveal prominence of eosinophils and perivascular fibrosis.

Dapsone is used as first-line therapy at doses of 50–100 mg orally each day. Topical, intralesional, or systemic glucocorticoids, chloroquine, colchicine, niacinamide, and sulfapyridine have also been used. An ophthalmology consultation should be considered (Wahl et al. 2005) to screen for ocular involvement.

Medium-Vessel Vasculitis

The most common dermatologic presentations of medium-vessel vasculitis include subcutaneous nodules, deep ulcers, and livedo reticularis. The skin lesions occur frequently in the lower extremities, primarily at dependent sites. Vasculitic nodules are typically painful and may ulcerate. Deep ulcers result from intense damage to blood vessel walls leading to reduced blood supply and ischemia. Livedo reticularis in medium-vessel vasculitis indicates interruption of blood flow in muscular arterioles in the deep dermis and subcutaneous tissue.

The major histopathologic features of medium-vessel vasculitis include inflammatory infiltrates infiltrating the muscular vessel wall and fibrin deposition within the vessel wall or lumen (with fibrinoid necrosis).

Polyarteritis Nodosa (PAN)

PAN is a systemic necrotizing vasculitis that mostly involves medium-sized muscular arteries. The incidence increases with age and the male-to-female ratio is reported to be 1.5:1. PAN is mostly idiopathic. Most cases of secondary PAN

are associated with HBV infection. The pathogenesis is not well understood but may include activation of the complement cascade (perhaps triggered by viruses) and immune complex deposition in the vessel wall. PAN usually presents with involvement of the kidneys, skin, joints, muscles, nerves, and/or gastrointestinal tract. Orchitis and coronary artery narrowing may occur in some patients. PAN spares the lung parenchyma. Cutaneous manifestations are observed in around 50 % of patients and may include painful and tender subcutaneous nodules, deep ulcers, and livedo reticularis. Bullous eruptions and palpable purpura may also be observed. Digital infarction and gangrene and large ulcers may occur in severe cases. The subcutaneous nodules in PAN follow the course of the superficial arteries in the lower extremities and may clinically mimic erythema nodosum. The nodules typically distribute in a livedoid pattern. The nodules usually resolve with atrophic, ivory-colored, retiform, stellate-shaped scars that may persist for years.

Approximately 10 % of patients with PAN present with predominately skin-related symptoms and milder systemic symptoms including fever, arthralgia, myalgia, and peripheral neuropathy (mononeuritis multiplex). These cases are considered cutaneous PAN. Cutaneous PAN is the most common form of PAN in the pediatric population. Cutaneous PAN is associated with streptococcal infection in children and has also been associated with infections by parvovirus B19, hepatitis B infection, and HIV.

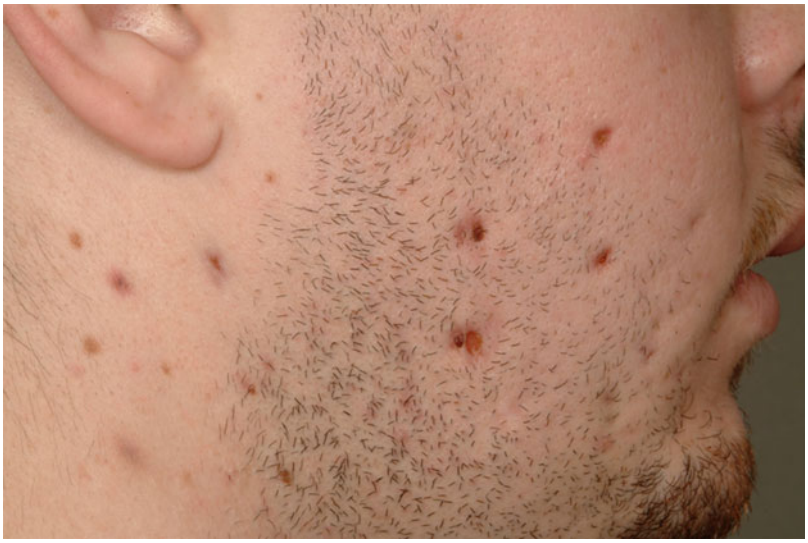
Whenever possible, a biopsy should be performed to confirm the diagnosis. Histopathologically, the biopsy from active subcutaneous nodules reveals a necrotizing arteritis with focal panniculitis surrounding the affected artery. This is in contrast with other forms of panniculitis that have inflammation in the septa, fat lobules, or have a mixed histologic picture, but without vasculitis.

Cutaneous PAN has a favorable prognosis, but exacerbations and relapses may occur. Patients with evidence of recent clinical or serologic streptococcal infection (such as an elevated ASO titer) should be treated with penicillin. Patients with cutaneous PAN frequently respond to dapsone or methotrexate. NSAIDs can be used to treat pain and mild exacerbations. Prednisone, with or without immunosuppressives, may be required for more severe or refractory disease. Prostaglandins (prostacyclin [PGI2]) and calcium channel blockers (nifedipine) have been tried for cases of cutaneous PAN presenting with digital infarction (Pagnoux et al. 2010).

Mixed (Small and Medium)-Vessel Vasculitides

Granulomatosis with Polyangiitis (Wegener's) (GPA)

GPA is a necrotizing, ANCA-associated vasculitis that primarily affects small- and medium-caliber vessels. GPA predominately affects older adults, is more common among Caucasians, and affects both genders equally. Its pathogenesis remains unknown. For example, it is not known whether ANCAs have a pathogenic role in GPA. Infections, genetic factors, and environmental factors, including medications have been proposed as the triggering factors. The activation of T cells, neutrophils, endothelial cells, and B cells may play a role. GPA typically presents with constitutional symptoms including anorexia, weight loss, fever, arthralgia, and myalgia. The most common organ involvement in GPA includes the ear, nose, throat, airways, lungs, kidneys, and/or skin. In addition to constitutional symptoms (such as fever and fatigue), the most common clinical symptoms include nasal crusting, purulent/bloody nasal discharge, oropharyngeal or nasal ulcerations, sinusitis, otitis media, hoarseness, cough, dyspnea, stridor, wheezing, and hemoptysis. Cutaneous manifestations are present in about one-half of patients with GPA, with skin lesions being the presenting feature in 10 % of cases. The activity of skin lesions often parallels that of systemic disease. The most frequent cutaneous manifestation is palpable purpura in the lower extremities. These purpuric lesions may be accompanied by focal necrosis and ulcers with corrugated borders resembling pyoderma gangrenosum.



Cutaneous Vasculitis, Fig. 2 Cutaneous manifestations are present in about one-half of patients with granulomatous polyangiitis (Wegener's) and may include papulonecrotic lesions or ulcerated papules

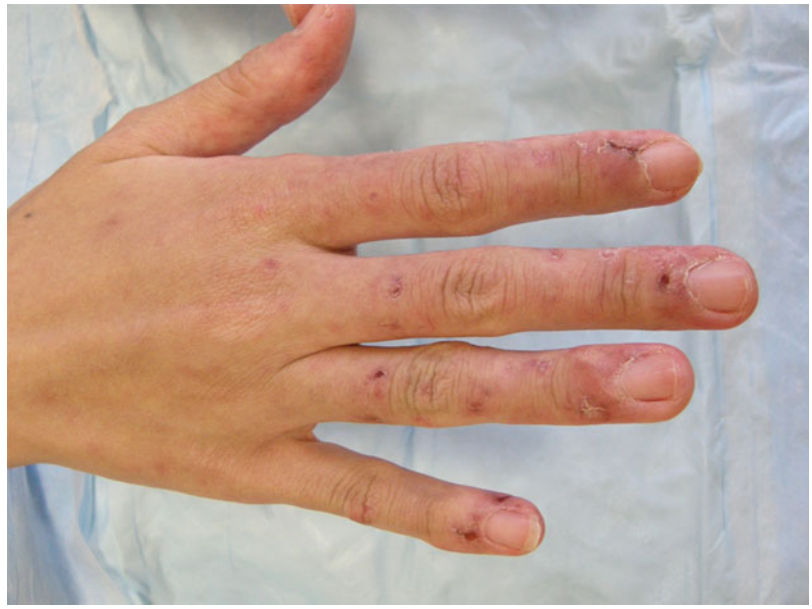
Papulonecrotic lesions, or ulcerated papules, may be present in 10 % of cases, typically favoring the elbows. Tender subcutaneous nodules, urticaria, livedo reticularis, vesicles, and splinter hemorrhages have also been observed in GPA (Fig. 2).

The diagnosis is suggested from the clinical manifestations and from the presence of circulating ANCA directed against proteinase-3 (PR-3). The absence of circulating ANCA does not exclude the diagnosis of GPA. The diagnosis of GPA should be confirmed by biopsy at a site of active disease, including skin. Skin biopsy reveals a leukocytoclastic granulomatous vasculitis with little or no immunoglobulin and complement on immunofluorescence. Biopsy of the kidney typically reveals a segmental necrotizing glomerulonephritis, with few or no immune deposits (pauci-immune) on immunofluorescence and electron microscopy. Calculated estimated glomerular filtration rate and urinalysis should be checked in all patients with GPA; while a kidney biopsy may not be required to establish the diagnosis, it can guide treatment. Granulomatous features may be seen on nasal biopsy and are occasionally diagnostic for GPA; however, nasal biopsies in suspected GPA frequently reveal nonspecific, necrotic tissue. A chest X-ray and computed tomography (CT) scan should be performed in all patients with suspected GPA.

Patients with GPA who are ANCA negative have a more favorable prognosis. Glucocorticoids, rituximab, methotrexate, cyclophosphamide, azathioprine and plasma exchange have been used for the therapy of GPA. Therapy depends on the organs involved, as well as the severity of the disease (Hoffman et al. 1992; Seo and Stone 2004).

Churg-Strauss Syndrome (CSS)

CSS is one of the ANCA-associated vasculitides that is characterized by a small- and medium-sized vessel vasculitis in association with asthma, chronic allergic rhinosinusitis, and eosinophilia. The median age at diagnosis is about 40 years. CSS tends to affect both genders equally. The pathogenesis of CSS, including the role of ANCAs, remains uncertain. There may be a role for abnormal immune function such as heightened Th1 and Th2 lymphocyte immunity, increased eosinophil recruitment, decreased eosinophil apoptosis, elevated serum IgE, and genetic factors such as HLA class and certain interleukin-10 polymorphisms. The onset of symptoms has been associated with several asthma medications, such as the leukotriene modifying agents, inhaled glucocorticoids, and omalizumab; however, CSS developing in patients taking these drugs appears to be due to unmasking of the underlying disease with the tapering of steroids rather than a causal



Cutaneous Vasculitis, Fig. 3 A subset of patients with SLE may have cutaneous vasculitis. Cutaneous vasculitis in SLE may present as digital ulcerations, microinfarctions, or subcutaneous nodules

relationship. The lungs and the skin are the most commonly involved organs in CSS. The prominent clinical features of the prodromal phase of CSS include poorly-controlled or late-onset asthma, allergic rhinitis, nasal polyposis and recurrent sinusitis. There is peripheral blood eosinophilia and eosinophilic infiltration of multiple organs, which can cause eosinophilic pneumonia, eosinophilic gastroenteritis, clinical signs of heart failure and cardiac rhythm abnormalities and peripheral neuropathy (mononeuritis multiplex) that may occur years later during the eosinophilic phase of CSS. Systemic vasculitis and granulomatosis typically occur in the vasculitic phase of CSS. About half to two-thirds of patients with CSS have skin involvement, particularly during the vasculitic phase of CSS. The most common cutaneous manifestations of CSS include palpable purpura, and tender subcutaneous nodules that favor the extensor surfaces of the arm, but can occur on the hands and legs, and scalp as well. Other skin lesions such as macular or papular erythematous rash, petechiae, extensive ecchymosis, papulonecrotic lesions, urticarial and livedo reticularis may also be observed in CSS.

The diagnosis is suggested by the presence of asthma, rhinosinusitis, and eosinophilia ($\geq$ 1,500 cells/microL and/or $>$ 10 % eosinophils on leukocyte differential). Biopsy of involved organs, including skin, lung, or peripheral nerve, can confirm the diagnosis. The major histopathologic features include leukocytoclastic vasculitis with eosinophilic infiltration, as well as interstitial and perivascular necrotizing granulomas. ANCA is positive in 40–60 % of patients, with most patients showing a peripheral staining pattern (p-ANCA) with specificity for myeloperoxidase (MPO).

The initial therapy for mild CSS is systemic glucocorticoids. Immunosuppressive agents such as azathioprine, methotrexate, mycophenolate mofetil, or cyclophosphamide may be added in patients with more severe disease (Sinico and Bottero 2009).

Cutaneous Vasculitis Secondary to Other Systemic Autoimmune Disease

A subset of patients with SLE may have cutaneous vasculitis (Fig. 3). Cutaneous vasculitis in SLE primarily affects small arterioles and postcapillary venules, most commonly presenting as urticaria and palpable purpura. Livedo reticularis, digital tip ulcerations or microinfarctions, subcutaneous nodules, or splinter hemorrhages in the nailfolds can also be seen. Other less common manifestations

include tender, erythematous, indurated lesions on the palms and the fingertips, chilblains (tender red to reddish-blue nodules on the fingers, toes, nose, or ears that occur as a reaction to cold temperatures), and atrophie blanche (ivory-colored scars with surrounding hyperpigmentation).

A small subset of patients with rheumatoid arthritis, particularly those with long-standing disease, a high level of rheumatoid factor, erosive joint disease, and rheumatoid nodules, may have rheumatoid vasculitis. Cutaneous vasculitis is the most common manifestation of rheumatoid vasculitis. Cutaneous small-vessel vasculitis presenting with palpable purpura and nailfold infarctions, appearing as periungual macules, may occur. In addition, necrotizing vasculitis of medium-caliber arteries presenting with deep ulcers on the lower extremities, typically in the area of the medial or lateral malleoli, or digital necrosis and gangrene may develop. Benign nailfold lesions, nodules on the finger pads, livedo reticularis, or urticaria may also be observed. It is important to rule out infection and vasculopathy. If a patient with rheumatoid arthritis and leg ulcers has skin biopsies that do not show vasculitis, pyoderma gangrenosum should be considered.

Cutaneous vasculitis in Sjogren's syndrome can present with palpable purpura, urticarial lesions, ecchymoses, or small ulcerations, usually on the lower extremities. Cutaneous vasculitis in Sjogren's syndrome is associated with antibodies to the Ro/SSA antigen. Sjogren's syndrome patients with cutaneous vasculitis are more likely to develop lymphoma. Vasculitis of medium-sized vessels presenting with large ulcers may be caused by cryoglobulinemia (Fiorentino 2003).

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Cross-References

- ▶ Cutaneous Vasculitis
- ▶ Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome)
- ▶ Neutrophilic Dermatoses: Leukocytoclastic Vasculitis
- ▶ Polyarteritis Nodosa
- ▶ Vasculitis: Granulomatosis with Polyangiitis (Wegener's)
- ▶ Vasculitis: Henoch-Schönlein Purpura

References

Black AK. Urticarial vasculitis. Clin Dermatol. 1999;17:565–9.

Carlson JA. The histological assessment of cutaneous vasculitis. Histopathology. 2010;56:3–23.

Carlson JA, Chen KR. Cutaneous vasculitis update: small vessel neutrophilic vasculitis syndromes. Am J Dermatopathol. 2006;28:486–506.

Carlson JA, Cavaliere LF, Grant-Kels JM. Cutaneous vasculitis: diagnosis and management. Clin Dermatol. 2006;24:414–29.

De Vita S, Soldano F, Isola M, Monti G, Gabrielli A, Tzioufas A, Ferri C, Ferraccioli GF, Quartuccio L, Corazza L, De Marchi G, Ramos Casals M, Voulgarelis M, Lenzi M, Saccardo F, Fraticelli P, Mascia MT, Sansonno D, Cacoub P, Tomsic M, Tavoni A, Pietrogrande M, Zignego AL, Scarpato S, Mazzaro C, Pioltelli P, Steinfeld S, Lamprecht P, Bombardieri S, Galli M. Preliminary classification criteria for the cryoglobulinaemic vasculitis. Ann Rheum Dis. 2011;70:1183–90.

Fiorentino DF. Cutaneous vasculitis. J Am Acad Dermatol. 2003;48:311–40.

Grzeszkiewicz TM, Fiorentino DF. Update on cutaneous vasculitis. Semin Cutan Med Surg. 2006;25:221–5.

Hautmann G, Campanile G, Lotti TM. The many faces of cutaneous vasculitis. Clin Dermatol. 1999;17:515–31.

Hoffman GS, Kerr GS, Leavitt RY, Hallahan CW, Lebovics RS, Travis WD, Rottem M, Fauci AS. Wegener's granulomatosis: an analysis of 158 patients. Ann Intern Med. 1992;116:488–98.

Hunder GG, Arend WP, Bloch DA, Calabrese LH, Fauci AS, Fries JF, Leavitt RY, Lie JT, Lightfoot Jr RW, Masi AT, et al. The American college of rheumatology 1990 criteria for the classification of vasculitis. Introduction. Arthritis Rheum. 1990;33:1065–7.

Jennette JC, Falk RJ. Small-vessel vasculitis. N Engl J Med. 1997;337:1512–23.

Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, Hagen EC, Hoffman GS, Hunder GG, Kallenberg CG, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum. 1994;37:187–92.

Pagnoux C, Seror R, Henegar C, Mahr A, Cohen P, Le Guern V, Bienvenu B, Mouthon L, Guillevin L, French Vasculitis Study Group. Clinical features and outcomes in 348 patients with polyarteritis nodosa: a systematic retrospective study of patients diagnosed between 1963 and 2005 and entered into the French Vasculitis Study Group Database. Arthritis Rheum. 2010;62:616–26.

Ramos-Casals M, Trejo O, García-Carrasco M, Cervera R, Font J. Mixed cryoglobulinemia: new concepts. Lupus. 2000;9:83–91.

Sais G, Vidaller A, Jucglà A, Gallardo F, Peyrí J. Colchicine in the treatment of cutaneous leukocytoclastic vasculitis. Results of a prospective, randomized controlled trial. Arch Dermatol. 1995;131:1399–402.

Saulsbury FT. Henoch-Schönlein purpura. Curr Opin Rheumatol. 2010;22:598–602.

Seo P, Stone JH. The antineutrophil cytoplasmic antibody-associated vasculitides. Am J Med. 2004;117:39–50.

Sinico RA, Bottero P. Churg-Strauss angiitis. Best Pract Res Clin Rheumatol. 2009;23:355–66.

Stone JH, Nousari HC. "Essential" cutaneous vasculitis: what every rheumatologist should know about vasculitis of the skin. Curr Opin Rheumatol. 2001;13:23–34.

Venzor J, Lee WL, Huston DP. Urticarial vasculitis. Clin Rev Allergy Immunol. 2002;23:201–16.

Wahl CE, Bouldin MB, Gibson LE. Erythema elevatum diutinum: clinical, histopathologic, and immunohistochemical characteristics of six patients. Am J Dermatopathol. 2005;27:397–400.

Xu LY, Esparza EM, Anadkat MJ, Crone KG, Brasington RD. Cutaneous manifestations of vasculitis. Semin Arthritis Rheum. 2009;38:348–60.

Cytotoxic T Lymphocytes

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Synonyms

CTL; Cytotoxic T lymphocytes; Killer T cell; Tc

Definition

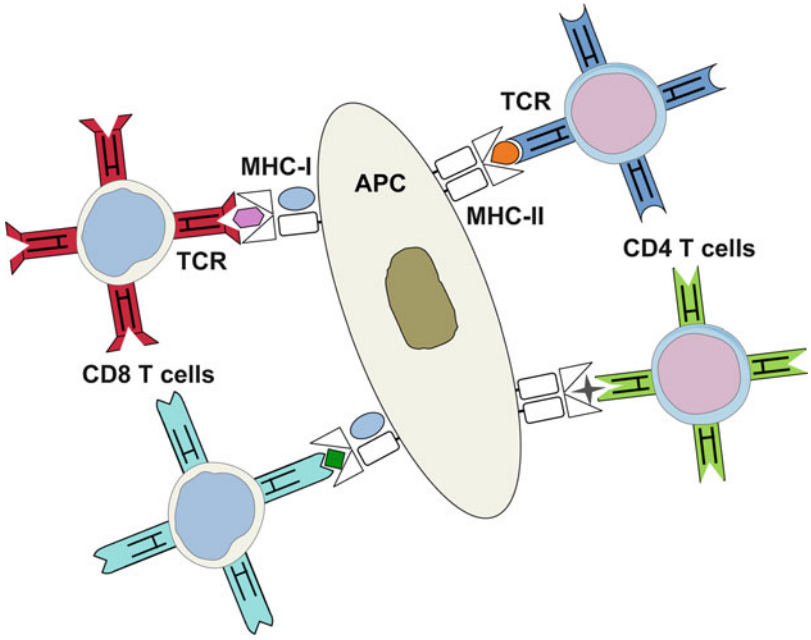
Cytotoxic T lymphocytes are specialized subsets of differentiated T cells which have the functional capability to kill target cells expressing nonself antigens. They are important components of the adaptive immune response and help control intracellular pathogens, as well as contain the development of tumors.

The Significance of Cytotoxic T Cells

Cytotoxic T lymphocytes (CTL) are a cornerstone of the adaptive immune response (Cox and Zajac 2010; Zhang and Bevan 2011). Due to their exquisite ability to detect and destroy cells presenting foreign antigens, they play a vital role in the initial control of many intracellular pathogens as well as help confer long-lived immunological protection against subsequent reinfections. Even in situations when the infection is not completely eradicated, such as during persistent chronic or latent infections, CTL function to dampen the initial burst of pathogen replication and then operate to either hold the infection at a steady state level or contain and limit the secondary spread of pathogens as they reactivate from latency. CTL responses also participate in tumor immunosurveillance and can limit the outgrowth of malignancies. CTL responses are not only induced by natural infections or as malignancies develop but are also generated in response to vaccines. Thus, CTL responses are versatile, durable, and vital for host defense.

CTL Recognition

The search and destroy functions of CTL are governed by the ability to co-recognize and respond to short peptide fragments expressed on the surface of the target cell in non-covalent association with either major histocompatibility complex (MHC) class I or MHC class II molecules (Fig. 1). CD8 T cells, which form the most prevalent population of CTL, usually



Cytotoxic T Lymphocytes, Fig. 1 *T cell recognition:* Each T cell expresses a unique, clonally distributed, TCR which governs their ability to recognize and respond to peptide-loaded MHC complexes. CD8 T cells bind to MHC class I–peptide complexes, whereas CD4 T cells recognize MHC class II–peptide complexes

recognize peptide fragments of 8–9 residues in length bound to MHC class I molecules (Neefjes et al. 2011). These antigenic peptides are most often derived from self and nonself (e.g., virus-derived) proteins which have been synthesized within the target cell and then subjected to proteolytic degradation. The antigen-processing machinery within the cell ensures that samples of these endogenous peptides associate with nascent MHC class I molecules and then localize to the cell surface for presentation to patrolling T cells. An alternative cross presentation pathway can also operate to enable peptides from exogenous proteins, which have been taken up by the target cells, to be presented by MHC class I molecules. Cytotoxic CD4 T cells are less common than CD8 CTL and their antigen-recognition properties are distinct. CD4 T cells recognize MHC class II complexes bound to peptide fragments which are generally slightly longer than those which associate with MHC class I molecules. Additionally, the peptides presented by MHC class II molecules are generally derived from exogenous antigens which have been endocytosed by specialized antigen-presenting cells (APCs) and then degraded. The resulting panels of peptides then assemble with MHC class II molecules and are presented at the cell surface. Notably, whereas MHC class I complexes are expressed by almost all cell types, the distribution of MHC class II molecules is largely confined to cells of the immune system, which limits the protective efficacy of CD4 CTL responses.

Which MHC–peptide complexes an individual T cell is capable of binding and responding to is determined by the precise sequence of the T cell's unique T cell receptor (TCR) (Bridgeman et al. 2012). The coding sequence, and thus exact structure, of the TCR is determined by a process of genetic recombination that favors diversification within the receptor regions that are most important for MHC–peptide interactions. This generates a tremendously diverse repertoire of TCRs. Nevertheless, TCRs are clonally expressed and thus an individual T cell will express a unique TCR that dictates its specific antigen and MHC recognition profile. At the population level, the lower limit of the number of T cells with nonidentical TCRs has been estimated to be 25×10^6. The actual repertoire is likely to be considerably greater, perhaps several thousandfold higher, because of additional sequence variation within the α- and β-chains which form the TCR complex

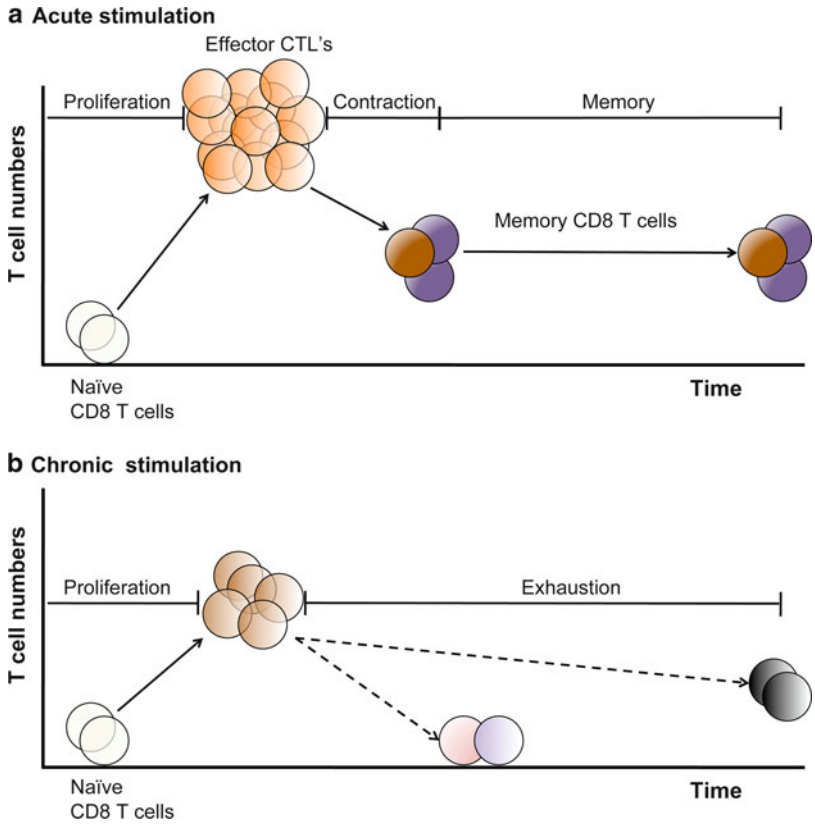
(Robins et al. 2009). This colossal inventory provides the host with a T cell pool with the potential power to detect and respond to the myriad of foreign antigens that it may encounter during its lifetime. Nevertheless, although the T cell repertoire is huge, the number of distinct naïve clones which recognize any given MHC–peptide complex is small. Estimates from murine studies have determined that there are perhaps several hundred individual T cells which express appropriate TCRs that allow them to respond to a specific peptide epitope.

Pathogens and other antigens, such as those associated with tumor development, are typically comprised of multiple epitopes which can potentially be recognized by distinct T cell clones. Nevertheless, not all epitopes are equally recognized and the magnitude, longevity, and efficacy of responses to individual epitopes varies, even if they are encoded by the same pathogen (Frelinger 2006). The hierarchical pattern of responses that emerge is determined by many factors including the properties of the antigen such as its stability, level of expression, and which cell types are presenting the antigen, as well as intrinsic T cell factors such as the avidity of the T cell for the MHC–peptide complex and the frequencies as well as activation states of the T cells which can respond to the specific antigen. The epitope hierarchy can influence the ability of CTL to control infections, as responses to individual epitopes can differ in their protective potential, and the pattern of immunodominance may change over time, during persistent infections, during recall responses, or as tumors evolve. Moreover, certain T cells may exhibit a degree of cross-reactivity which may confer beneficial additional immunological protection or cause detrimental immunopathology.

Priming the CTL Response

CTL responses are induced following an antigen-driven activation process which typically results in massive T cell expansion, their differentiation and acquisition of effector functions, and the disbursement of the cells throughout the host (Fig. 2a) (Cox and Zajac 2010; Sheridan and Lefrancois 2011). The overall response is typically comprised of CD4 helper T cells, CD8 CTL, which form the major CTL population, and under certain conditions a smaller number of CD4 CTL. Pronounced CD8 T cell responses have been detected during many infections including influenza virus, vaccinia virus, Epstein-Barr virus (EBV), yellow-fever virus, lymphocytic choriomeningitis virus (LCMV), and early following human immunodeficiency virus (HIV) infection. Moreover, it is becoming increasingly well appreciated that the vast majority of CD8 T cells detected at the peak of the response are pathogen specific (Zhang and Bevan 2011).

Usually the T cell responses are triggered in secondary lymphoid organs – the spleen and lymph nodes – as naïve T cells encounter professional APCs and recognize MHC–peptide complexes. This interaction between the T cells TCR and its cognate-presented antigen is the first signal that begins the subsequent clonal expansion and cellular differentiation; however, this initial interaction alone is insufficient to sustain the response. During infections, APCs also become activated as part of the host's innate defense mechanism, leading to the upregulation of many surface receptors including the costimulatory molecules CD80 and CD86. In order to prevent aberrant immune responses to innocuous or self-antigens, T cells must also receive costimulation via CD28 interacting with CD80 or CD86 on the APC. This second signal promotes T cell survival and a burst of interleukin (IL)-2 production. In addition, other costimulatory molecules, including many members of the tumor necrosis factor (TNF) receptor superfamily, can also influence the ensuing response. Infections with pathogens also elicit the production of other immunological warning signs including proinflammatory cytokines such as IL-12, antiviral factors such as type I interferons, and alarmins such as IL-33. All of these elements can serve as important third signals for driving the full activation of the T cell response and amplifying effector activities (Curtsinger and Mescher 2010). As the small number of naïve T cells detect antigen (signal 1),



Cytotoxic T Lymphocytes, Fig. 2 *The phases of the CTL response:* (**a**) Acute antigenic exposure, in conjunction with costimulatory signals and inflammatory cues, can drive massive proliferation and differentiation of CD8 T cells. This process ensures that an expanded pool of effector T cells form which operate to clear the inducing antigen. As the antigen is cleared, a contraction phase ensues during which most of the highly functional, terminally differentiated, T cells succumb to apoptosis. At the conclusion of the contraction phase, a pool of memory CD8 T cells remain and are represented at a greater frequency than their naïve counterparts. These memory populations can be stably maintained for years following priming and function to confer long-lived immunological protection. (**b**) Aberrant T cell responses develop under conditions of persistent antigenic stimulation, such as during chronic viral infections. Typically the burst size of the response is smaller than that observed following acute stimulation. Although the responding cells develop certain effector traits and can attain a highly activated terminally differentiated phenotype, a spectrum of functional defects can arise. As effector activities are gradually extinguished, the antigen-specific T cells may not be stably maintained

engage costimulatory molecules (signal 2), and sense certain soluble factors (signal 3), they are driven into cell cycle, proliferate vigorously, and form a highly differentiated effector pool.

During the proliferative phase of the response, the properties of the responding T cells profoundly change. The expression of adhesion molecules, as well as cytokine and chemokine receptors, shifts allowing the cells to migrate from the sites of priming in secondary lymphoid organs to other locations where they may encounter infected cells (Cox and Zajac 2010; Sheridan and Lefrancois 2011). Metabolic changes also occur which are necessary for the rapid and substantial cell division that the T cell will undergo. Importantly, the responding cells differentiate into effector cells with the ability to rapidly produce numerous cytokines as well as kill target cells. These effector activities are triggered as T cells detect and bind to their cognate MHC–peptide complexes on target cells and can be initiated by the newly differentiated

effector cell without the requirement for costimulation or third signals. The CTL population is, however, functionally heterogeneous with certain T cells more polyfunctional and capable of eliciting a broad range of effector activities, whereas other constituents of the effector pool may possess more limited functional abilities (Makedonas and Betts 2006).

Most of the CD8 T cells that are initially generated become terminally differentiated effector CTL as the response develops. If the inducing antigen is controlled, then the vast majority of these terminally differentiated T cells undergo apoptosis. Ideally the response does not completely collapse. Instead, as homeostasis is restored, a population of memory T cells remain present (Fig. 2a). Memory T cells can be maintained at remarkably stable levels and have been detected for decades following smallpox vaccination. Maintaining this memory population contributes to immunological protection against reexposure to antigen or reinfection with the pathogen. Although there are far fewer antigen-specific T cells present during the memory phase than at the peak of the response, the frequency of these cells is higher than at the onset of the priming event. Moreover, by comparison with their naïve counterparts, memory T cells are generally tuned to mount faster and more efficacious responses upon antigenic activation.

Memory T cells are distinct from their naïve and effector counterparts, and they are also diverse (Lanzavecchia and Sallusto 2005). A subset of memory cells which express lower levels of the adhesion molecule CD62L and the chemokine receptor CCR7 are referred to as effector memory cells. They are preferentially located in non-lymphoid tissues where they are immediately available to rapidly elicit effector activities, including cytokine production and cytotoxicity, in response to reexposure to antigen at these sites. They are sensitive to MHC–antigen activation but do not require professional APC or other signals in order to function. These tissue resident cells are often retained at the site of the primary infection, such as in the lung following influenza infection or the skin and dorsal root ganglion following herpes simplex virus infection, but can be more systemically distributed (Sheridan and Lefrancois 2011). A population of memory T cells, termed central memory T cells (T_{CM}), express higher levels of CD62L and CCR7 and are primarily present in secondary lymphoid organs. In some ways, T_{CM} display a less differentiated phenotype than effector memory T cells and are more prone to proliferate upon secondary stimulation. Although T_{CM} may be less efficient at deploying immediate effector activities, they can attain these traits as they begin to divide in response to antigenic activation.

The induction of robust effector responses and the development of T cell memory are usually associated with antigens that are cleared from the host, which occurs, for example, following vaccination or during acute infections. A spectrum of functional and phenotypic differences in T cell responses have been observed under conditions of antigen persistence which develop as a result of the failure to eradicate the infection or tumor (Fig. 2b) (Yi et al. 2010). The production of cytokines including interferon (IFN)-γ, TNF-α, and IL-2, as well as cytotoxic effector molecules such as perforin and granzymes, may be diminished or abolished as the T cells responding to these persistent stimuli fail to attain or lose polyfunctional traits and succumb to exhaustion. Differences in proliferative capacity and dependency on homeostatic cytokines such as IL-7 and IL-15 have also been observed, and severe functional exhaustion can culminate in the physical loss of antiviral CD8 T cells. Comparative analysis of CD8 T cell responses to viral infections which result in different levels of antigenic exposure, such as influenza virus, cytomegalovirus, EBV, hepatitis C virus, and HIV, indicates that antiviral CD8 T cells may adopt different preferred phenotypic and functional set points. This is influenced by the level and repetitiveness of the antigenic stimuli, as well as by the balance of activatory and suppressive cytokines, and also by the presence of helper and regulatory CD4 T cells.

Transcriptional Control of CTL Differentiation

The profound phenotypic and functional changes that the responding T cells undergo during the priming phase reflect modifications in gene expression which are regulated by transcription factors (Cox et al. 2011; Kallies 2008). Inflammatory cytokines such as IL-12 and IFN-γ induce expression of the transcription factor T-bet (*tbx21*), which promotes the differentiation of type I helper CD4 T cells as well as CD8 CTL responses. High levels of T-bet drive the terminal differentiation of effector CTL, which have potent cytolytic capacity but limited proliferative abilities. Notably, if CD4 T cell help is insufficient, then T-bet levels are high, which promotes effector CD8 T cell development but curbs the formation of effective memory responses. The related transcription factors FOXO1 and FOXO3 also regulate effector T cells and in the case of FOXO1, this is due to inhibition of T-bet which restricts the differentiation and expansion of the response. Lower amounts of T-bet, however, permit the formation of memory T cells, which can be maintained over time and give rise to secondary effector cells during recall responses. In mice, T-bet deficiency results in reduced effector CTL and favors the development of long-lived memory T cells. Nevertheless, some effector phenotype cells are still generated, and some cytotoxic potential is still retained as a result of compensation by another transcription factor, eomesodermin (Eomes). Eomes expression is most commonly associated with memory T cell formation, and this transcription factor is repressed by the inflammatory conditions that induce T-bet. Deletion of Eomes alone does not prevent development of an effector CTL pool; however, these cells are poorly maintained as memory cells and appear incapable of responding to secondary challenge. If both T-bet and Eomes are deleted from CD8 T cells, however, the CTL response is aberrant and infection control compromised.

In addition to T-bet and Eomes, the transcription factor B-lymphocyte-induced maturation protein (Blimp)-1 (*prdm1*) is also critical for CTL development. The absence of Blimp-1 results in poor expression of the cytolytic molecules perforin and granzyme B and poor control of viral infections. One function of Blimp-1 may be to induce greater levels of T-bet and reduce Eomes expression, thereby favoring effector CTL development. In Blimp-1-deficient T cells, *tbx21* expression is lower, and *eomes* expression is significantly higher at the mRNA level. Similar to T-bet, the level of Blimp-1 expressed in a cell is critical to determining the functional capacity of CD8 T cells. Therefore, controlling the amount of Blimp-1 expressed within a responding CTL molds that cell's ultimate fate and function.

The related transcription factors Id2 and Id3 support T cell survival and promote memory formation. Interestingly, Blimp-1 suppresses Id3, which likely helps to cement the development of terminally differentiated effector T cells at the expense of memory formation. Moreover, Bcl-6, a transcription factor associated with memory T cells, can also repress Blimp-1 expression. Overexpression of Bcl-6 drives expansion of memory T cells, and the effector cells generated under these conditions display reduced killing and granzyme B expression. As Blimp-1 and Bcl-6 are mutual repressors, this increase in Bcl-6 expression may be preventing the Blimp-1-driven differentiation of the effector response that ordinarily occurs following T cell activation.

Antigenic activation is the principle driver of the T cell response, but the levels of cytokines, which flux during the course of many infections and immune responses, also regulate the T cell transcriptional network (Cox et al. 2011). As discussed above, T-bet levels are determined in part by inflammatory cytokines such as IL-12. IL-2 signaling in CD8 T cells strongly induce the expression of Eomes and Blimp-1. Moreover, signaling through the IL-10 and IL-21 cytokine receptors activates the transcription factor STAT-3 which influences the expression of Blimp-1 and Bcl-6. Hence, the composition of the cytokine milieu affects the phenotypic and functional attributes attained by the responding T cells.

CTL Effector Mechanisms

Since CTL recognize and respond to MHC–peptide complexes expressed upon the cell surface, cell–cell contact is necessary for the elaboration of their effector activities. Thus, by comparison with antibody responses, CTL cannot confer sterilizing immunity and prevent infections by neutralizing the cell-free form of the pathogens present in circulation or other body fluids. CTL responses do, however, provide an important cellular mechanism for eliminating pathogen-infected cells or other cell types expressing nonself or tumor-associated antigens. For lytic infections, the rapidity of CTL response is critical as swift action by these cells limits the release of the pathogen and eliminates the infected cell before full replicative potential is reached. For non-lytic infections, which can parasitize the target cell without causing its destruction, CTL function to destroy these factories of pathogen production.

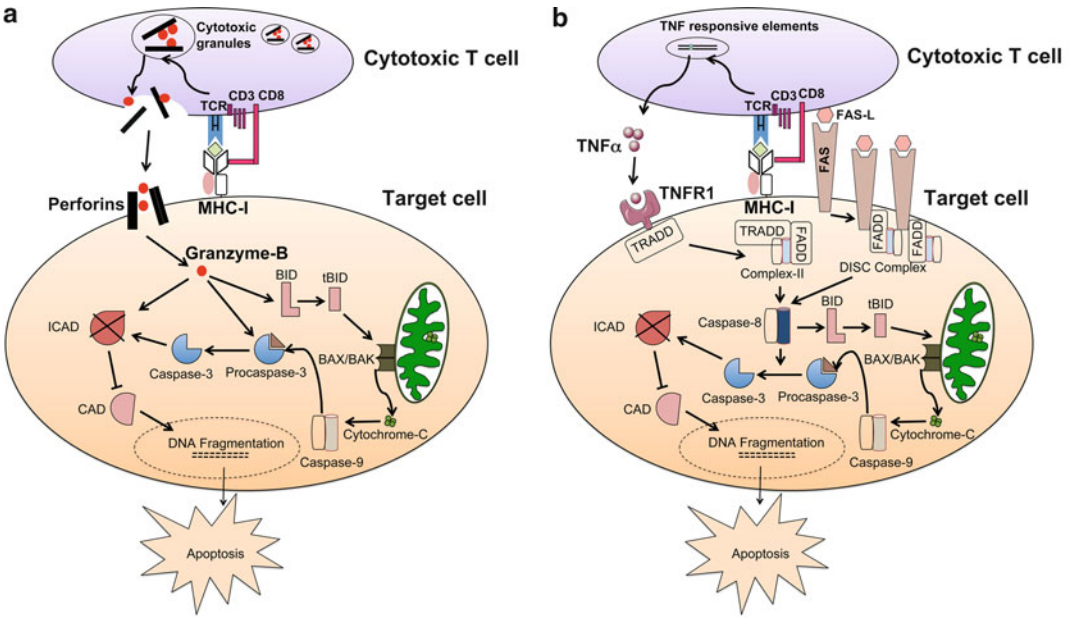
CTL functions are commonly associated with terminally differentiated effector cells which possess cytolytic functions and can also express a variety of cytokines and chemokines (Makedonas and Betts 2006). The secretion of effector cytokines and chemokines is initiated following contact of the T cell with a target cell and in the case of IFN-γ requires new protein synthesis. The production of cytokines such as IFN-γ and TNF-α by T cells can mediate the clearance of certain infections including hepatitis B virus without causing target cell death (Guidotti and Chisari 2001). Conversely, in the case of influenza infection, cytolytic effector functions can be elaborated against pulmonary epithelial cells without activating cytokine production. Such multifunctional capabilities provide a degree of flexibility and functional breadth necessary to combat diverse intracellular pathogens and tumors.

Perforin/Granzyme-Mediated Cytotoxicity

The best studied and most common mechanism of CTL-induced cell death is perforin- and granzyme-mediated cytotoxicity (Fig. 3a). As CTL gain their effector capabilities, they develop cytolytic granules which contain the pore-forming protein perforin and a variety of granzymes. As these effector CTL recognize target cells via their TCR, an immunological synapse forms between the engaged cells (Jenkins and Griffiths 2010). At the time of target cell engagement, preexisting perforin is the first to be released as marked cytoskeletal rearrangements occur within the CTL and the lytic granules migrate along microtubules toward the immunological synapse where they are discharged. This activation process can also stimulate the production of new perforin which replenishes the CTL and sustains effector capabilities.

Upon release perforin polymerizes in the target cell membrane permitting entry of granzymes which deliver the "kiss of death" to the target cell. In addition, at high concentrations, granzymes can access target cells in a perforin-independent manner. Although CTL express a variety of granzymes, the roles of granzyme B in the lytic process are most well defined (Ewen et al. 2012). Granzyme B has many molecular targets including BH3-interacting domain death agonist (BID), procaspase-3, and inhibitor of caspase-activated DNase (ICAD). Granzyme B cleaves BID into its truncated form, tBID and procaspase-3 into activated caspase-3. tBID translocates into the outer mitochondrial membrane and promotes the oligomerization of Bax and Bak, leading to membrane disruption and the release of cytochrome C into the cytosol. The cytochrome C release activates caspase-9, which further activates caspase-3, ultimately resulting in the cleavage of ICAD, which can also be directly acted upon by granzyme B, into caspase-activated DNase (CAD). Once activated, CAD then enters the nucleus causing DNA fragmentation and apoptosis.

The mechanisms of action for other granzyme molecules (A, H, K, and M) are less well understood (Ewen et al. 2012). Notably, patients with a variety of inflammatory conditions express detectable levels of soluble granzymes, which may have non-cytotoxic biological roles. It has been shown that granzyme A can elicit the production of inflammatory cytokines.



Cytotoxic T Lymphocytes, Fig. 3 *CTL killing mechanisms.* (**a**) Perforin/granzyme-mediated cytotoxicity occurs as CTL engage a target cell via their TCR, relaying a signal that releases prestored cytotoxic granules containing perforin and granzymes. Perforin forms a pore in the target cell membrane which enables the entry of granzymes. Granzyme B functions as a key inducer of apoptosis, resulting in target cell destruction. (**b**) CTL can also cause target cell apoptosis by activating the death receptor pathway via FasL–Fas interactions or by the production of TNF-α. Engagement of Fas on the target cell by FasL, which is expressed on certain populations of CTL, launches a signaling cascade via formation of the DISC complex which ultimately results in apoptosis. TNF-α, which is produced by certain CTL subsets, also causes death receptor-mediated apoptosis following binding to its receptor TNFR1 and recruiting TRADD, leading to the formation of complex II. Signaling through complex II activates downstream executioner caspases leading to cell death

In addition, granzyme A and its family member granzyme K are able to induce cell death independently of caspase activity and the release of cytochrome C from mitochondria. Granzymes A and K are also both capable of inducing the production of reactive oxygen species which can cause cell death. In addition to production of reactive oxygen species, granzyme K can also activate mitochondrial apoptosis pathways, as measured by cytochrome C release.

Throughout the killing process, the CTL must avoid autolysis and self-destruction by their own lytic granules. In order to preserve their own integrity, CTL encode the protease inhibitor-9 (in humans) or its murine ortholog, serine protease inhibitor-6, which functions to stabilize the granules within the CTL and also protects against granzyme B-mediated apoptosis (Ashton-Rickardt 2010). In addition, at the time of granule deployment, CTL release cathepsin-B which prevents perforin from attacking the effector T cell.

Death Receptor-Mediated Apoptosis

Although perforin/granzyme-dependent cytotoxicity is a principal effector mechanism, CTL can also mediate death receptor apoptosis via the Fas ligand (FasL)–Fas or TNF receptor pathways (Fig. 3b) (Smyth and Trapani 1998). The FasL–Fas pathway is initiated, as activated CTL upregulate FasL on their cell surface, usually following engagement with a target cell. The interaction between FasL on the CTL and Fas on the surface of the target cell promotes the formation of the death-inducing signaling complex (DISC), which is comprised of Fas-associated death domain (FADD), procaspase-8, and procaspase-10. Signaling through DISC results in cleavage of these

procaspases, releasing the active caspases, which in turn activate caspase-3. Caspase-3, as an executioner caspase, then cleaves ICAD, activating CAD, thus inducing DNA fragmentation and cell death. Caspase-8 also cleaves BID into tBID, which oligomerizes Bax and Bak, causing the release of cytochrome C from the mitochondria. This release of cytochrome C activates caspase-9 and elicits a downstream signaling pathway which also culminates in cell death.

TNF-α can be secreted by CTL upon antigenic stimulation and is also a mediator of cell death. TNF-α binding to TNF receptor 1 (TNFR1) on the target cell results in trimerization of the receptor complex and recruitment of TNFR-associated death domain (TRADD). FADD is also recruited forming complex II, which leads to caspase activation and induction of cell death through DISC, paralleling Fas-mediated apoptosis. TNF-α-induced cell death can also be mediated by membrane-bound TNF-α, independently of the secreted cytokine.

CTL-mediated cell death is usually a tightly regulated and exquisitely antigen-dependent process. Nevertheless, CTL can sometimes cause apoptosis in bystander cells that are not directly recognized by the effector cell. This can result from FasL present on the CTL surface engaging Fas-expressing target cells in the absence of TCR signaling. Similarly, cells expressing TNFR1 may be susceptible to bystander killing caused by membrane-bound or secreted TNF.

Evading the CTL Response

The power of CTL responses in combating pathogens is well illustrated by the various strategies which have evolved to counteract the actions of the effector response (Keckler 2007). Many pathogens encode specific molecules which interfere with antigen-processing and presentation pathways, thereby circumventing T cell recognition. In addition, an effective CTL response can exert pressure on the pathogen or tumor to select for viable genetic variants with escape mutations within antigenic epitopes. Such alterations can render the existing population of CTL ineffective if they cannot recognize or efficiently respond to the variant pathogen or tumor. Pathogens have also adopted immune evasion strategies which directly interfere with the CTL-mediated killing process. For example, certain viruses encode serpins that block caspase activation, which is critical for the cytolytic process. These molecules include one of the most potent inhibitors of caspase-mediated cell death, CrmA, encoded by cowpox virus, as well as related serpins encoded by vaccinia virus, myxoma virus, and ectromelia virus. Granzyme B is directly inhibited by CrmA, and this death pathway is also targeted by the herpes simplex virus glycoprotein J and the adenovirus-encoded L4-100K protein. Death receptor signaling is blocked by vFLIP and related molecules encoded by herpesviruses and poxviruses, which disrupt the formation and function of the DISC, impeding the induction of apoptosis. Fas-mediated cell death is blocked by the adenovirus E3 region-encoded receptor internalization and degradation complex, as well as by the myxoma virus LAP protein which reduces surface expression of this death receptor. Soluble TNF decoy receptors encoded by poxviruses also directly block the action of this cytokine.

Cytotoxic CD4 T cells

CD4 T cells are most commonly associated with helper functions which promote B cell and CD8 T cell responses and with regulatory properties which can suppress the development of pathogenic immune responses. CD4 T cells can, however, also adopt a cytotoxic phenotype, and cytolytic CD4 T cell responses have been detected following several infections including influenza virus, West Nile virus, hepatitis C virus, human cytomegalovirus, HIV, EBV, and LCMV (Marshall and Swain 2011). The effector mechanisms used by CD4 CTL mirror those used by conventional CD8 CTL. Like their CD8+ counterparts, CD4 CTL have multifunctional properties and can express cytokines including TNF-α and IFN-γ as well as cytolytic effector molecules including perforin,

granzymes, FasL, and, in humans, granulysin. CD4 CTL are detected in both lymphoid and non-lymphoid organs and may display a terminally differentiated phenotype, similar to the highly cytolytic effector CD8 CTL, (CCR7-, CD27-, CD28-, CD62L-, CD45RO+), and may express the senescence marker CD57.

CD4 CTL recognize peptide–MHC class II complexes which are primarily expressed by professional APC, but under inflammatory conditions expression can be induced on certain other cell types including fibroblasts and endothelial cells. Therefore, by comparison with CD8 T cells, which recognize broadly expressed MHC class I complexes, the more limited tissue distribution of MHC class II molecules likely limits the efficacy of the CD4 CTL response. Nevertheless, CD4 CTL may be important during EBV infection or B cell lymphocytic leukemia, as they are able to recognize and kill infected or malignant MHC II-expressing B cells. In addition, early cytolytic CD4 responses are associated with better control of HIV-1 infection and under certain circumstances CD4 CTL can also have a pathogenic role.

Immunopathology

Although CTL play a vital protective role, by clearing infections, eliminating tumors, or limiting the levels of pathogen replication, there can be detrimental consequences to these responses. Since CTL can kill target cells and produce inflammatory cytokines, they can cause tissue damage. Such immunopathology is classically described following infection of mice with the non-cytopathic virus LCMV. CD8 CTL responses are essential for the clearance of this infection. This effector response can also cause the death of the infected animal, an outcome which depends upon the route of infection, the age of the mouse, the levels of virus replication, and the tempo of the T cell response. During hepatitis B and C virus infections, virus-specific T cells infiltrate the liver, causing destruction of hepatocytes, leading to liver cirrhosis. The extent of liver injury coincides with the number of virus-specific CTL present in the liver, and pathology can be mollified by depleting hepatitis-specific CTL at the peak of the infection. This immunopathology is attributed to the cytopathic activity of the CTL, although secretion of effector cytokines and chemokines can recruit other inflammatory cells into the liver, enhancing the magnitude of tissue damage. Interestingly, during many chronic infections such as viral hepatitis or HIV, CTL lose function as they succumb to exhaustion (Yi et al. 2010). This rebalancing of the effector response may limit the ability to control the infection but also may serve as a strategy to curtail immunopathology.

Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Adaptive Immune Cells in the Liver
- ▶ Animal Models of Hepatitis B and C
- ▶ B7 and CD28 Families
- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Fas/Fas Ligand
- ▶ Immune Responses to the Hepatitis C Virus
- ▶ Mammalian Target of Rapamycin (mTOR)
- ▶ T Cell Memory
- ▶ Tumor-Infiltrating T Cells

References

Ashton-Rickardt PG. Serine protease inhibitors and cytotoxic T lymphocytes. Immunol Rev. 2010;235:147–58.

Bridgeman JS, Sewell AK, Miles JJ, Price DA, Cole DK. Structural and biophysical determinants of alpha beta T-cell antigen recognition. Immunology. 2012;135:9–18.

Cox MA, Zajac AJ. Shaping successful and unsuccessful CD8 T cell responses following infection. J Biomed Biotechnol. 2010;159–152.

Cox MA, Harrington LE, Zajac AJ. Cytokines and the inception of CD8 T cell responses. Trends Immunol. 2011;32:180–6.

Curtsinger JM, Mescher MF. Inflammatory cytokines as a third signal for T cell activation. Curr Opin Immunol. 2010;22:333–40.

Ewen CL, Kane KP, Bleackley RC. A quarter century of granzymes. Cell Death Differ. 2012;19:28–35.

Frelinger J. Immunodominance – The choice of the immune system. Weinheim: Wiley-VCH; 2006.

Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol. 2001;19:65–91.

Jenkins MR, Griffiths GM. The synapse and cytolytic machinery of cytotoxic T cells. Curr Opin Immunol. 2010;22:308–13.

Kallies A. Distinct regulation of effector and memory T-cell differentiation. Immunol Cell Biol. 2008;86:325–32.

Keckler MS. Dodging the CTL response: viral evasion of Fas and granzyme induced apoptosis. Front Biosci. 2007;12:725–32.

Lanzavecchia A, Sallusto F. Understanding the generation and function of memory T cell subsets. Curr Opin Immunol. 2005;17:326–32.

Makedonas G, Betts MR. Polyfunctional analysis of human T cell responses: importance in vaccine immunogenicity and natural infection. Springer Semin Immunopathol. 2006;28:209–19.

Marshall NB, Swain SL. Cytotoxic CD4 T cells in antiviral immunity. J Biomed Biotechnol. 2011;954602.

Neefjes J, Jongsma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol. 2011;11:823–36.

Robins HS, Campregher PV, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, Riddell SR, Warren EH, Carlson CS. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood. 2009;114:4099–107.

Sheridan BS, Lefrancois L. Regional and mucosal memory T cells. Nat Immunol. 2011;12:485–91.

Smyth MJ, Trapani JA. The relative role of lymphocyte granule exocytosis versus death receptor-mediated cytotoxicity in viral pathophysiology. J Virol. 1998;72:1–9.

Yi JS, Cox MA, Zajac AJ. T-cell exhaustion: characteristics, causes and conversion. Immunology. 2010;129:474–81.

Zhang N, Bevan MJ. CD8+ T cells: foot soldiers of the immune system. Immunity. 2011;35:161–8.

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Dendritic Cells in Atherosclerosis

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Definition

Dendritic Cells are a subset of myeloid leukocytes that play key roles in initiating and directing the adaptive immune response. Dendritic cells, which were named based on their branch-like morphology ("déndron" meaning "tree" in Greek), can be found at multiple sites of non-lymphoid tissues and within lymph nodes, and are characterized by their ability to migrate through lymphatic vessels and present antigens as professional antigen presenting cells. Dendritic cells are also detected within non-diseased and atherosclerotic aortas, but the functions of DC subsets in the development and progression of atherosclerosis are not well understood.

Historical Perspective

Dendritic cells were discovered by Ralph Steinman and Zanvil Cohn in 1973 (Steinman 2011). Initially, DCs have been described as cells that are highly mobile and, in contrast to macrophages, are weakly phagocytotic. These newly discovered cells had a large nucleus, and abundant cytoplasm, arranged in processes of different length and width. In 1978, using a mixed leukocyte reaction, it was demonstrated that DCs are potent stimulating cells that are at least 100 times more effective than other major cell subclasses in activating T cells. To date, DCs are found in various tissues of the body where they constantly communicate with a range of lymphocyte populations to serve as a bridge between the external environment and the adaptive immune system (Ueno et al. 2007). DCs capture antigens in peripheral tissues and then migrate to secondary lymphoid organs, including lymph nodes and the spleen, in order to present antigen to naïve T cells (Mempel et al. 2004). During the migration to secondary lymphoid organs, DCs also receive additional Toll-like receptor (TLR)-dependent or Nod-like receptor-dependent stimulation signals that result in DC maturation. The maturation of DCs is accompanied by the upregulation of MHC-II, the co-stimulatory molecules CD80 and CD86, and the chemokine receptor CCR7. Dendritic cells (DCs) are potent antigen-presenting cells that present various endogenous and exogenous antigens to T cells (Coquerelle and Moser 2010; Ueno et al. 2007). Antigen presentation results in either initiation of the adaptive immune response or in the induction of tolerance and the release of anti-inflammatory factors. There are 4 major categories of DCs: conventional DCs that are specialized in antigen processing and presentation; Langerhans cells; plasmacytoid DCs; and monocyte-derived DCs, which are induced in response to inflammation.

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Vascular Dendritic Cells in Healthy Aortas

Dendritic cells are found within healthy human and mouse aortas (Galkina and Ley 2009; Hansson and Hermansson 2011). Human aortic DCs express $CD1a^+S\text{-}100^+lag^+$ and resemble Langerhans cells (Bobryshev 2010). These vascular-associated DCs are termed as vascular DCs. Despite the fact that some macrophages and monocytes express CD11c; the expression of the CD11c integrin is a key marker for DC identification (Geissmann et al. 2010a). Bone marrow-derived $CD11c^+CD68^+$ cells are found within the lesion-susceptible lesser curvature of the healthy aortic intima of C57BL/6 mice. This site-specific localization of intimal $CD11c^+$ cells occurs independently from circulating cholesterol levels, suggesting an important role of the microenvironment at specific anatomical locations. DCs are also found within the cardiac valve and aortic sinus of C57BL/6 mice. These aortic DCs express low levels of CD40 and are positive for CD1d, CD80, and CD86, suggesting an immature phenotype. Recent studies have also highlighted the complexity of the origins of DCs and their functions within the aorta (Bobryshev 2010; Koltsova and Ley 2011; Niessner and Weyand 2010). DCs are generated at least by two major pathways that differ in their requirement for the Flt3/Flt3L (Flt3L, fms-like tyrosine kinase 3 ligand) axis (Geissmann et al. 2010b). The development of DCs from monocyte-independent precursors is Flt-3/Flt3L-dependent, whereas the generation of DCs from monocytes is Flt3/Flt3L-independent (Geissmann et al. 2010b). A recent study demonstrated that Flt3 treatment leads to an expansion of $CD11c^+$ cells within the intima and adventitia indicating at least a partial monocyte-independent origin of some $CD11c^+$ DCs under healthy, non-atherosclerotic conditions. $CD11c^+$ cells that are located within the healthy murine aorta do not have a uniform phenotype and represent at least two major populations: $CD11c^+CD11b^+F4/80^+$ and $CD11c^+CD11b^-F4/80^-$ DCs. Additional studies have demonstrated that these two DC populations originate from different precursors. $CD11c^+CD11b^+F4/80^+CD103^-$ DCs are M-CSF-dependent and are likely derived from a common monocyte precursor, whereas $CD11c^+CD11b^-F4/80^-CD103^+$ DCs are Flt3-dependent and are derived from DC precursors (Table 1).

One of many remaining unresolved questions concerns the functions of DCs within the

Dendritic Cells in Atherosclerosis, Table 1 Vascular dendritic cell subset in atherosclerosis

Vascular DC subset	Effects on murine atherosclerosis	Marker profile
Flt-3-dependent DCs, derived from common DC precursors	Depletion of Flt-3 increases aortic plaques and Th1 cell content, but decreases Tregs in Ldlr−/− mice	CD11c+CD11b-CD103+
Flt-3 independent, M-CSF-dependent monocyte-derived DCs	Unknown	CD11c+MHC-II+CD11b+F4/80+DC-SIGN+CD103-
GM-CSF-dependent DCs	Unknown	CD11c+CD11b-CD68+MHC-II+
CCL17+ DCs	DC-derived CCL17 limits the expansion of Tregs and increases atherosclerotic plaques	CD11c+CD11b+F4/80-CCL17+
Plasmacytoid DCs	Functions are controversial. Depletion of pDCs (anti-PDCA-1 Abs) increases aortic plaques in Ldlr−/− mice, whereas pDCs-derived IFNα induces TRAIL-1-dependent VSMC apoptosis	CD11clowIFNα+CD11b-

This table describes known subsets of vascular dendritic cells and their functions during development and progression of atherosclerosis

Abbreviations: DCs dendritic cells, *GM-CSF* granulocytes-macrophage colony-stimulating factor, *CCL17* chemokine (C-C motif) ligand 17, *Flt3* fms-like tyrosine kinase 3, *Th1* T helper 1, *Treg* T regulatory cells, *$Ldlr^{-/-}$ mice* low density lipoprotein receptor deficient mice

non-diseased aortic wall. It might be possible that resident DCs provide local tolerance and regulate the balance between the active and suppressor arms of the immune response. However, recent data also suggest a potential pro-atherogenic role for several subsets of DCs in atherosclerosis. A specific subset of aortic resident DCs ($CD11c^+CD11b^-$ DCs) uptake neutral lipids in high-cholesterol diet-fed $Ldlr^{-/-}$ mice within the atherosclerosis-prone areas of the lesser curvature (Cybulsky and Jongstra-Bilen 2010). An important question concerning DC functions in atherosclerosis is whether these professional antigen-presenting cells are capable of presenting antigens within the aortic wall. Several studies using OT-I and OT-II TCR-transgenic mice (Miyagawa et al. 2010) that are specific for OT-I and OT-II OVA peptide have demonstrated that aortic $CD11c^+$ DCs are able to present OVA peptide to OT-I and OT-II T cells in vitro and in vivo. Importantly, these results correlate with experiments conducted using a model of engineered bioartificial human arteries that mimic the size and structural dimensions of human arteries. Human $CD11c^+$ DCs seeded within bioartificial arteries migrated to the intima, where they initiated an adaptive immune response, suggesting that antigen presentation by human DCs can occur in vivo (Niessner and Weyand 2010).

Dendritic Cells in Atherosclerotic Aortas

Accumulation of DC within the Aorta

Atherosclerosis-prone conditions induce significant recruitment of multiple populations of immune cells including monocytes, mast cells, neutrophils, and T cells into the aortic wall and surrounding adventitia (Galkina and Ley 2007). An increased number of $CD11c^+$ cells are found in close proximity to T cells in the zones of neovascularization within murine and human atherosclerotic plaques. DCs are detected in the shoulder regions of unstable human plaques. There are several potential mechanisms by which DCs might accumulate within the aortic wall (Ley et al. 2011). Atherosclerosis-prone conditions increase the number of peripheral blood-circulating proinflammatory monocytes that might be converted at a higher rate to aortic DCs. The direct recruitment of pre-DCs to the aorta may impact the total aortic $CD11c^+$ DC content.

The migration of monocytes and pre-DCs is directed by specific adhesion molecules and aortic chemokines, produced by endothelial and stromal cells, and subsets of leukocytes (Galkina and Ley 2007; Woollard and Geissmann 2010). P- and E-selectins play an important role in monocyte rolling along the endothelium, and combined deficiency in P- and E-selectins decreases atherosclerosis by 80 % in atherosclerosis-prone $Ldlr^{-/-}$ mice. Evidence from VCAM-1 or α_4 integrin blockade experiments suggests that VCAM-1 supports the slow rolling and tight adhesion of monocytes to the atherosclerotic endothelium. CXCL1 and CCL5, either alone or as a heterodimer with CXCL4, serve as arrest chemokines to initiate integrin activation and firm monocyte adhesion to endothelium. CXCL7 and migration inhibitory factor (MIF) also efficiently trigger monocyte arrest to the inflamed endothelium under flow conditions. CCL2 is known to be one of the key chemokines in monocyte biology. Classical $CCR2^+$ monocytes exit the bone marrow in a CCL2-dependent manner, and both CCL2 and CCL7 maintain monocyte homeostasis in circulation (Serbina and Pamer 2006). Several studies suggest that the CCL2/CCR2 axis participates in atherogenesis by the modulation of monocyte recruitment into the aorta, mostly likely via regulation of monocyte transmigration. An increase in DC content correlates with the progression of atherosclerosis with simultaneous elevation in both subsets of aortic $CD11c^+$MHC-II^+CD11b^+ Flt-3-independent DCs and $CD11c^+$MHC-II^+CD11b^- Flt3-dependent DCs in the atherosclerotic aortas of $Ldlr^{-/-}$ mice (Koltsova and Ley 2011). Increased aortic DC numbers may not be due to increased migration from peripheral circulation alone, but due to reduced emigration out of the vessel wall or increased local proliferation as well. For example, a short-term high-cholesterol diet feeding elevated local

proliferation of intimal $CD11c^+CD11b^-$MHC-II^+ DCs in the aorta of $Ldlr^{-/-}$ mice. Several lines of evidence also demonstrate defective egress of macrophages from atherosclerosis-prone aortas. Similarly, defective aortic DC egress from the aorta might be a possible reason for DC accumulation within the atherosclerotic vessel.

Possible Functions of Dendritic Cells During Atherogenesis

The accumulation of DCs within the atherosclerotic aorta induces the proinflammatory status of DCs and supports lesion progression (Koltsova and Ley 2011). Despite the attenuated ability of DCs to migrate from peripheral tissues to draining lymph nodes during atherogenesis, DCs isolated from $Ldlr^{-/-}$ or $Apoe^{-/-}$ mice efficiently induce T cell proliferation and IFNγ and TNFα production after ex vivo coculture with naïve $CD4^+$ T cells (Koltsova and Ley 2011). DCs are specialized in capturing, processing, and presenting antigen-derived peptides on major histocompatibility complex (MHC) molecules in vivo, thereby, allowing DCs to shape T cell responses in order to modulate immunity or tolerance (Steinman 2011). Several modifications of low density lipoproteins (LDL) affect the maturation and activation of DCs. Oxidized phospholipids (ox-PLs) alter DC activation and prevent their maturation through the blockade of TLR-3- and TLR-4-dependent induction of CD40, CD80, CD83, and CD86. In contrast, oxidized LDL (oxLDL) support the maturation of DCs that secrete IL-12 but not IL-10 and support both syngeneic and allogeneic T cell stimulations (Galkina and Ley 2009).

There are several potential antigens including oxLDL, beta-2 glycoprotein I (β2GPI), and heat shock proteins HSP-60 and HSP-65 that might induce the Th1-specific response during atherogenesis. Interestingly, analysis of T cell hybridomas from human apolipoprotein B100 transgenic mice that were immunized with human oxLDL revealed that T cell clones surprisingly respond to native and purified LDL apolipoprotein ApoB100, but not to oxLDL, suggesting the existence of an immune response against native LDL (Hansson and Hermansson 2011).

Direct evidence for the implication of DCs in atherosclerosis was obtained using mice that overexpress the anti-apoptotic gene hBcl-2 under a CD11c promoter (Galkina and Ley 2009; Hansson and Hermansson 2011). The content of $CD11c^{high}$MHC-II^{high} DCs was increased in DC-hBcl2 mice and was accompanied with enhanced T cell activation, elevated levels of Th1, Th17-related cytokines, and increased production of Th1-driven IgG2c antibodies, suggesting that $CD11c^+$ DCs are involved in T cell activation and Th1/Th17 helper differentiation in vivo. Interestingly, DC-hBcl2 mice also demonstrate reduced levels of plasma cholesterol. Depletion of CD11c in CD11c-diphtheria toxin receptor (DTR) (Apolipoprotein E-deficient) $Apoe^{-/-}$ transgenic mice results in elevated levels of circulating cholesterol highlighting a unique ability of DCs to modulate cholesterol levels in blood (Koltsova and Ley 2011). Further studies are necessary to provide better understanding of the mechanisms of the regulation of circulating cholesterol by DCs.

The function of multiple DC subsets in atherosclerosis is not well understood; however, several recent studies provide strong evidence for subset-specific functions of DCs. $CD11c^+CD11b^-CD103^+$ cells are Flt-3-dependent subset of DCs that induce tolerance in the steady state. Constitutive ablation of these DCs results in autoimmune disease. Flt-3 deficiency and concomitant depletion of $CD11c^+CD11b^-CD103^+$ DCs resulted in increased plaque burden within the aortic sinus and the aortic arch, and a shift toward the Th1 response at the cost of the Treg population in $Flt\text{-}3^{-/-}Ldlr^{-/-}$ mice (Choi et al. 2011). Thus, aortic $CD11c^+CD103^+$ DCs likely function as tolerogenic DCs during atherosclerosis. In contrast to $CD11c^+CD103^+$ DCs, another subset of DCs that expresses CCL17 reduces Treg expansion and supports atherogenesis. The number of Tregs decreases proportionally to the progression of atherosclerosis, but the mechanisms behind this reduction remain unclear. The discovery of suppressor $CD11c^+CD103^+$ DCs and

proinflammatory CCL17$^+$ DCs provides new understanding for the role of different DC subsets in the regulation of the immune response during atherogenesis.

There are several lines of evidence suggesting that plasmacytoid DCs (pDCs) might play a role in atherosclerosis. The hallmark of pDCs is the rapid production of high levels of type I interferon (interferon α/β, IFN) and other cytokines in response to TLR7, TLR8, and TLR9 engagement. Additionally, pDCs express low levels of CD11c and are positive for some lymphocyte antigens, including CD4, Rag-1, and CD45RA/B220 (Reizis et al. 2011). Since pDCs are weak antigen-presenting cells compared to conventional DCs, the major function of pDCs is to provide fine-tune for the balanced T helper responses via production of type I interferons. IFNα$^+$ DCs are found within atherosclerosis-prone human and mouse vessels (Koltsova and Ley 2011; Niessner and Weyand 2010). The role of pDCs in atherosclerosis is not completely understood. pDCs-derived type I IFN induces the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on CD4$^+$ T cells that initiate VSMCs apoptosis, suggesting an important link between pDC and vascular inflammation. On the other hand, pDCs might serve as an immunogenic cell sentinel and provide suppressor functions via expression of tolerogenic enzyme indoleamine 2,3-dioxygenase (IDO), the inducible costimulator ligand (ICOS-L), and/or the programmed death 1 ligand (PD-L1), which mediates Treg development. In line with this concept, the depletion of pDCs increases the plaque burden in *Ldlr*$^{-/-}$ recipient mice with simultaneous elevated T cell proliferation and IFNγ production, suggesting a tolerogenic suppressor role (Koltsova and Ley 2011). Thus, the involvement of pDCs in atherosclerosis remains controversial and further studies are necessary to delineate the functions of pDCs in atherogenesis.

DCs and Vaccination

DCs regulate the T and B cell responses and are often targets and vectors for vaccination. It appears that DC subsets and different methods of DC activation might elicit different T cell responses. In addition to the complexity of the involvement of different DC subsets in atherosclerosis, there is also a question about the specific antigens to target. One of the first approaches to test whether vaccination might modulate atherogenesis utilized immunization of rabbits and mice with oxLDL (de Jager and Kuiper 2011). This approach resulted in attenuated atherosclerosis. Further studies using DC-based vaccination strategies have demonstrated different outcomes. The injection of bone marrow-derived DCs pulsed with oxLDL prior to lesion formation attenuated lesion formation in the brachiocephalic artery after collar placement. Multiple subcutaneous injections of bone marrow-derived DCs pulsed with malondialdehyde (MDA)-LDL or keyhole limpet hemocyanin (KLH)-loaded DCs led to an elevated plaque burden within the aortic roots. Apolipoprotein B100 (ApoB100) is a protein component of LDL, and the progression of atherosclerosis is associated with immune responses to ApoB100-derived peptides. Interestingly, immunization of mice expressing human ApoB100 in the liver with bone marrow-derived LPS maturated DCs treated with IL-10 and ApoB100 before the development of atherosclerosis reduces atherosclerosis in recipient mice after Western diet feeding. Thus, DCs with induced tolerogenic properties might be one of the promising approaches to modulate the T cell response and attenuate chronic inflammation of the aortic wall that accompanies atherogenesis (de Jager and Kuiper 2011).

Conclusion

Since the discovery of DCs within the aorta in 1995, our understanding of DC functions during the development and progression of atherosclerosis has significantly advanced. Multiple studies using gene-deficient and transgenic mice have demonstrated that atherosclerosis reduction correlates with decreased number of CD11c$^+$ DCs. Additionally, several studies have also highlighted significant heterogeneity of DC

subsets within the aorta and uncovered potential complexity of DC functions during atherogenesis. DCs have the unique capacity to discriminate between self and nonself, and are therefore well suited to regulate the delicate balance between immunity and tolerance. The identification of the factors that determine the functional activity of specific DC subsets will allow designing new approaches for atherosclerosis treatment.

Cross-References

- ▶ Atherosclerosis and Cytokines
- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Dendritic Cells in Atherosclerosis
- ▶ Lymphocytes in Atherosclerosis
- ▶ Macrophages, Oxidative Stress, and Atherosclerosis
- ▶ Normal Immune Function and Barrier: Langerhans Cells
- ▶ Tregs in the Liver

References

Bobryshev YV. Dendritic cells and their role in atherogenesis. Lab Invest. 2010;90:970–84.
Choi JH, Cheong C, Dandamudi DB, Park CG, Rodriguez A, Mehandru S, Velinzon K, Jung IH, Yoo JY, Oh GT, Steinman RM. Flt3 Signaling-dependent dendritic cells protect against atherosclerosis. Immunity. 2011;35:819–31.
Coquerelle C, Moser M. DC subsets in positive and negative regulation of immunity. Immunol Rev. 2010;234:317–34.
Cybulsky MI, Jongstra-Bilen J. Resident intimal dendritic cells and the initiation of atherosclerosis. Curr Opin Lipidol. 2010;21:397–403.
de Jager SC, Kuiper J. Vaccination strategies in atherosclerosis. Thromb Haemost. 2011;106:796–803.
Galkina E, Ley K. Leukocyte influx in atherosclerosis. Curr Drug Targets. 2007;8:1239–48.
Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis (*). Annu Rev Immunol. 2009;27:165–97.
Geissmann F, Gordon S, Hume DA, Mowat AM, Randolph GJ. Unravelling mononuclear phagocyte heterogeneity. Nat Rev Immunol. 2010a;10:453–60.
Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science. 2010b;327:656–61.
Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol. 2011;12:204–12.
Koltsova EK, Ley K. How dendritic cells shape atherosclerosis. Trends Immunol. 2011;32:540–7.
Ley K, Miller YI, Hedrick CC. Monocyte and macrophage dynamics during atherogenesis. Arterioscler Thromb Vasc Biol. 2011;31:1506–16.
Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature. 2004;427:154–9.
Miyagawa F, Gutermuth J, Zhang H, Katz SI. The use of mouse models to better understand mechanisms of autoimmunity and tolerance. J Autoimmun. 2010;35:192–8.
Niessner A, Weyand CM. Dendritic cells in atherosclerotic disease. Clin Immunol. 2010;134:25–32.
Reizis B, Bunin A, Ghosh HS, Lewis KL, Sisirak V. Plasmacytoid dendritic cells: recent progress and open questions. Annu Rev Immunol. 2011;29:163–83.
Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. Nat Immunol. 2006;7:311–7.
Steinman RM. Decisions about dendritic cells: past, present, and future. Annu Rev Immunol. 2011;30:1–22.
Ueno H, Klechevsky E, Morita R, Aspord C, Cao T, Matsui T, Di PT, Connolly J, Fay JW, Pascual V, Palucka AK, Banchereau J. Dendritic cell subsets in health and disease. Immunol Rev. 2007;219:118–42.
Woollard KJ, Geissmann F. Monocytes in atherosclerosis: subsets and functions. Nat Rev Cardiol. 2010;7:77–86.

Dermatomyositis, Scalp

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Synonyms

Connective tissue disease; Inflammatory myopathy

Definition

Dermatomyositis is an uncommon inflammatory disease characterized by muscle weakness and a distinctive skin rash. It affects adults and children alike. In adults, dermatomyositis usually

occurs from the late 40s to early 60s; in children, the disease most often appears between 5 and 15 years of age. Dermatomyositis affects more females than males.

Introduction

Dermatomyositis (DM) is a systemic autoimmune connective tissue disease with characteristic cutaneous and muscle inflammation. It is classified as adult or juvenile idiopathic DM, amyopathic DM, or associated with internal malignancy.

Epidemiology

The incidence of dermatomyositis and polymyositis (DM/PM) is estimated at more than 5.5 cases per million. There is a female predominance with female-to-male ratio of 2:1. In patients aged 50 and above, it has a strong association with malignancy.

Pathogenesis

The precise pathogenic mechanism of DM remains uncertain. Features of superficial vasculopathy on histology suggest a capillary pathology. Clinically vasculopathy is common in childhood DM and is seen in more severe disease (Silver and Maricq 1989). Cutaneous ulceration in adults due to vasculopathy is a poor prognostic indicator (Tomb and Stephan 2002). Angiogenesis, leukocyte trafficking, and complement cascade associated genes are highly unregulated, and dendritic cells are highly enriched at the perivascular inflammatory sites. This suggests neovascularization and endothelial cell activation in both juvenile and adult DM. It is likely this close association of monocytes with endothelial cells initiates rapid dendritic cell maturation and an autoimmune response in DM (Nagaraju et al. 2006). A strong correlation with HLA-DR7 has been demonstrated (Costner and Grau 2006).

Clinical Findings

The characteristic cutaneous features of DM are the heliotrope rash and Gottron's papules. Other cutaneous findings are malar erythema, poikiloderma overexposed skin such as the extensor surfaces of the arms, the "V" of the neck, or the upper back (shawl sign), periungual telangiectasia, bullous and erosive lesions, hyperkeratosis of the lateral fingers and palms (mechanic's hands), panniculitis, urticaria, and exfoliative dermatitis. Scalp involvement in DM is often overlooked and initially misdiagnosed. Early in its course, it may mimic psoriasis, seborrheic dermatitis, or contact dermatitis. Scalp disease manifests as diffuse, confluent, atrophic, violaceous, and scaly plaques. The diagnosis of DM should be considered in both adult and pediatric patients with scaly scalp and other cutaneous and systemic signs of DM (Kasteler and Callen 1994; McDonald and Smith 1998). Peloro et al. in a retrospective study identified 16 patients under 18 years of age with juvenile DM, and 25 % of these had psoriasiform scalp dermatitis at the time of diagnosis (Peloro et al. 2001). Hypertrichosis of face and extremities is also seen in many patients with juvenile DM (Pope et al. 1994).

Kasteler and Callen in a study in 1994 showed scalp involvement in DM as a relatively common finding. In this study, 82 % patients with diagnosis of DM had scalp involvement in the form of diffuse, erythematous, scaly, atrophic, and psoriasiform dermatitis, often misdiagnosed as psoriasis or seborrheic dermatitis. Mild to moderate alopecia was noted in 43 % patients and was of a nonscarring nature. In some patients, scalp or cutaneous disease without systemic involvement of DM can be the presenting complaint. This is termed as "amyopathic DM" or "DM sine myositis." In such cases, a scalp/skin biopsy is helpful. No correlation between scalp involvement and muscle disease was noted in this study (Kasteler and Callen 1994). Another study by Tilstra JS et al. showed scalp involvement in 63 % of their patients with diagnosis of DM and 33 % patients had associated nonscarring alopecia (Tilstra et al. 2009). Nonscarring alopecia often follows a flare of the systemic disease. Lesions of DM are

frequently intensely pruritic, which is a differentiating characteristic from lupus erythematosus. Scalp pruritus can be particularly challenging to manage. Rarely, DM itself causes cicatricial alopecia, but it may frequently be associated with other connective tissue diseases like lupus which can result in cicatricial alopecia. The striking violaceous color of DM rash helps to differentiate it from the pinkish red hue of the rash seen in lupus. Also, the rash of DM is more pruritic than that of lupus.

During disease flare-up, patients can experience more hair shedding due to telogen effluvium. It is a nonscarring, diffuse hair loss in which an increased number of hairs go into resting phase (telogen phase). A hair pull test is positive (with more than six hairs out of group of 60 hairs coming out) easily with an elongated hair bulb visible to naked eye. Telogen effluvium can be due to disease activity or due to systemic medications used to treat the disease.

Diagnosis

The diagnosis of DM is suspected in patients with classic clinical findings and confirmed by a skin biopsy and serological tests. In patients with only scalp involvement, a scalp biopsy will be helpful to exclude other dermatoses. Biopsy specimen taken from scaly erythematous plaque may be indistinguishable from cutaneous lupus erythematosus. However, Crowson and Margo have suggested that microvascular pathology may enable distinction of DM from systemic lupus erythematosus (SLE) and subacute cutaneous lupus erythematosus (SCLE) (Crowson and Magro 1996).

Histopathology

Scalp biopsy shows same findings such as skin biopsy including epidermal atrophy, hyperkeratosis, follicular plugging, and vacuolar interface changes often with PAS positive irregular thickening of basement membrane zone. Subtle dermal mucin deposition and dermal edema with sparse perivascular lymphocytic infiltrate may be seen.

Immunofluorescence microscopy shows deposition of immunoglobulin at the dermo-epidermal junction, but of much less intensity than seen in lupus. Deposition of complements C5–C9 is a significant feature of DM.

Serologic Tests

Serologic tests include antinuclear antibody (ANA) testing which is positive in one-third to one-half of patients and do not correlate with disease activity (Parodi et al. 2002). Numerous myositis-specific antibodies have been recognized including Anti-Jo-1, anti Mi-2, anti-Ku, 140/155. Recently, melanoma differentiation–associated gene 5 (MDA-5) has been identified as a DM-specific auto-antigen that appears to be targeted in patients with DM and mild or absent muscle inflammation and with an increased risk of interstitial lung disease. MDA-5 has been associated with increase in scalp involvement and hair loss (Fiorentino et al. 2011).

Treatment

Cutaneous or scalp disease may remain persistent even after the myositis and systemic disease of DM are controlled with corticosteroids or immunosuppressive therapy. Daily use of broad-spectrum sunscreen with sun protective clothing must be recommended to all patients with skin or scalp disease. Anti-pruritic therapies are often necessary along with topical therapy corticosteroids or calcineurin inhibitors, such as tacrolimus or pimecrolimus. Intralesional corticosteroids (triamcinolone acetonide 10 mg/ml) every 4 weeks can reduce inflammation and pruritus.

In more severe cases, hydroxychloroquine 200–400 mg/day is effective. Chloroquine phosphate 250–500 mg/day and/or quinacrine 100 mg once or twice daily can be recommended for patients who do not respond to hydroxychloroquine.

In a study by Zieglschmid et al. and Kasteler and Callen, methotrexate at a dose of 15–35 mg/week was found to be useful for skin lesions. Other medications reported to be effective in cutaneous disease are mycophenolate mofetil, thalidomide, leflunomide, anti-estrogens, oral tacrolimus, IVIG, infliximab, sirolimus, and rituximab.

Cross-References

- ▶ Dermatomyositis, Skin
- ▶ Lichen Planus

References

Costner MI, Grau RH. Update on connective tissue diseases in dermatology. Semin Cutan Med Surg. 2006;25(4):207–20.

Crowson AN, Magro CM. The role of microvascular injury in the pathogenesis of cutaneous lesions of dermatomyositis. Hum Pathol. 1996;27(1):15–9.

Fiorentino D, et al. The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to MDA5 (CADM-140): a retrospective study. J Am Acad Dermatol. 2011;65(1):25–34.

Kasteler JS, Callen JP. Scalp involvement in dermatomyositis. Often overlooked or misdiagnosed. JAMA. 1994;272(24):1939–41.

McDonald LL, Smith ML. Diagnostic dilemmas in pediatric/adolescent dermatology: scaly scalp. J Pediatr Health Care. 1998;12(2):80–4.

Nagaraju K, et al. Endothelial cell activation and neovascularization are prominent in dermatomyositis. J Autoimmune Dis. 2006;3:2.

Parodi A, et al. Dermatomyositis in 132 patients with different clinical subtypes: cutaneous signs, constitutional symptoms and circulating antibodies. Acta Derm Venereol. 2002;82(1):48–51.

Peloro TM, et al. Juvenile dermatomyositis: a retrospective review of a 30-year experience. J Am Acad Dermatol. 2001;45(1):28–34.

Pope DN, Strimling RB, Mallory SB. Hypertrichosis in juvenile dermatomyositis. J Am Acad Dermatol. 1994;31(2 Pt 2):383–7.

Silver RM, Maricq HR. Childhood dermatomyositis: serial microvascular studies. Pediatrics. 1989;83(2):278–83.

Tilstra JS, et al. Scalp dermatomyositis revisited. Arch Dermatol. 2009;145(9):1062–3.

Tomb R, Stephan F. Perforating skin ulcers occurring in an adult with dermatomyositis. Ann Dermatol Venereol. 2002;129(12):1383–5.

Dermatomyositis, Skin

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Synonyms

Amyopathic dermatomyositis; Dermatomyositis sine myositis; Idiopathic inflammatory myopathy

Definition

Dermatomyositis is a progressive connective tissue disorder characterized by inflammatory and degenerative changes of the muscles and skin.

Clinical Presentation

The cutaneous manifestations of dermatomyositis are arguably the most distinct and dramatic of any other autoimmune connective tissue disease. Once recognized, the clinician may establish the diagnosis even when there is histologic as well as serologic ambiguity. The unique quality of the rash is as important as its typical location pattern. Lesions typically present as patches or plaques, and all are characterized by either pink or violaceous erythema that can be with or without scale. The heliotrope eruption of the eyelids, as is typical for most of the cutaneous findings in dermatomyositis, is very distinctive and may be associated with persistent periocular edema. However, it may initially be thought to be contact dermatitis. The malar rash targets the nasolabial folds in contrast to lupus where they are spared. As rosacea does the same, initial diagnoses of rosacea are often considered in these patients. With absence of pustules, a unique type of blush rosacea does in fact occur. V-sign and Shawl sign

occur in the majority of patients which over time may lead to a poikilodermatous appearance. Linear extensor erythema often hides underlying Gottron's sign and/or papules over the extensor IP joints of the hands. The latter may be found over the elbows and knees, sometimes mimicking psoriasis. Periungual suffusion with cuticular disarray and overgrowth, and vessel "dropout" associated with a striking corkscrew pattern is found. Mechanics hand and the MDA-5 related palmar papules along the creases may also be found and initially considered as contact dermatitis or eczema. Pocket or holster sign in the vicinity of the trochanteric bursa on the lateral upper thighs is unique and often puzzling when presenting alone. Ulcerations may occur early in the MDA-5 subtype or late in association with disabling calcinosis cutis. Scalp involvement is common and described in the autoimmune hair loss section.

Amyopathic dermatomyositis (dermatomyositis sine myositis) has been shown to have the same incidence of interstitial pulmonary fibrosis as well as associated malignancy as the myositic type. A novel autoantibody to a 155-kd protein distinct from the anti-synthetases, is associated with cancer-associated dermatomyositis with or without muscle involvement.

Pathogenesis

Dermatomyositis skin disease is characterized by several pathologic findings: vacuolar interface dermatitis associated with keratinocyte (KC) injury/apoptosis; a cellular infiltrate consisting of largely T lymphocytes, dendritic cells, and macrophages located in a perivascular distribution, at the dermo-epidermal junction (DEJ), and occasionally in a peri-adnexal distribution; increased deposition of mucin in the papillary and (usually) reticular dermis; papillary dermal vascular ectasia; evidence of vasculopathy consisting of endothelial cell injury and/or vascular fibrin deposition and thrombi (Smith et al. 2009). Current data regarding the role of the immune system and environment in these pathogenic events are reviewed below.

Immune System Dysregulation

Cell Types Seen in DM Skin

Several investigators have shown that T lymphocytes (both CD4+ and CD8+) dominate the cellular infiltrate in DM skin (Caproni et al. 2004; Wenzel et al. 2006; Magro et al. 2010). In general, CD4+ lymphocytes are found primarily in a perivascular distribution, and more abundant than the CD8+ lymphocytes that are often located at the DEJ (Caproni et al. 2004; Magro et al. 2010). This has led to the postulation that keratinocyte injury might be mediated by cytotoxic lymphocytes, although this has not been shown. In addition, neutrophils and plasmacytoid dendritic cells (pDC) are commonly found in DM skin (Caproni et al. 2004; Wenzel et al. 2006; Magro et al. 2010). Interestingly, pDC are found mainly at the DEJ and perivascularly rather than in the deep dermis as is seen in cutaneous lupus (McNiff and Kaplan 2008). CD68+ cells are also found in high abundance, whereas B cells are rarely seen (Caproni et al. 2004; Dourmishev and Wollina 2006; Magro et al. 2010). Mast cells are also found in DM skin, even in skin that is uninvolved, suggesting that they may be critical in initiating DM skin disease (Caproni et al. 2004; Shrestha et al. 2010).

There are little data regarding the antigen specificity of the T lymphocytes in DM skin. The clear association of DM with MHC class II polymorphisms suggests that the adaptive T cell response is critical to disease pathogenesis (Robinson and Reed 2011). Many of them appear to be activated (as they express MHC class II and CD40L) although it is unclear if they are part of an antigen-driven response in the skin (Caproni et al. 2004; Dourmishev and Wollina 2006). T lymphocytes might be driven by upregulation of adhesion molecules such as VCAM and ICAM on the vessels, which is found in DM skin (Hausmann et al. 1996). Additionally, IFN gene products could act as chemoattractants (see below). Th1 cells appear to predominate over Th2 cells (Caproni et al. 2004; Wenzel et al. 2006; Magro et al. 2010), although the role of other Th types (e.g., Th17) has not been

investigated to date. It is tempting to speculate that the T cells are responding to many of the antigens that are known to be targeted by circulating autoantibodies in DM, although very little data currently exist regarding antigen expression in DM skin. Mi2 is known to be expressed in mouse keratinocytes, but it is unclear how this relates to DM skin (Kashiwagi et al. 2007). A recent publication demonstrated that the MDA5 antigen is expressed at high levels in DM skin (mainly in KC), although the identity of the putative cellular target(s) of this immune response in the skin is at present unknown (Zahn et al. 2011).

It has recently been shown that Treg cells are found at a decreased frequency in DM skin compared to many other inflammatory disorders (Solomon and Magro 2008; Antiga et al. 2010; Magro et al. 2010). Both FoxP3$^+$ and TGF-beta-expressing cells are found at a lower density in DM skin compared to other inflammatory disorders. This suggests that the initiation and/or propagation of T cell activation in DM skin may be in part due to defects in negative regulation of T cells.

Interferon (IFN)

As in DM muscle and peripheral blood, DM skin is characterized by overexpression of a large number of IFN-induced transcripts and proteins (Wenzel et al. 2005, 2006; Wong et al. 2012). A global transcriptional study of DM skin demonstrated that this high level of IFN-induced transcripts is seen also in cutaneous lupus and herpetic infections, and, to a lesser degree, in psoriasis (Wong et al. 2012). It has been suggested that this high IFN "signature" is characteristic of any type of interface dermatitis that is characterized by KC injury (Wenzel and Tuting 2008). Wenzel and colleagues suggest that IFN-induced proteins such as CXCL9 and CXCL10 act as chemoattractants for CXCR3-bearing lymphocytes that then mediated KC injury (Wenzel et al. 2006). Some data suggest that MxA (another IFN-induced protein) levels correlate with the number of CXCR3$^+$ cells in DM skin biopsies (Wenzel et al. 2006). In addition, IFNs are known to upregulate genes critical for immunoregulation, activate dendritic cells and natural killer cells, and mediate T cell activation and survival. Although it has been suggested that type I IFN is mediating these effects, the exact contribution of the various IFN subtypes in DM skin is not yet known. One possible source of type I IFN would be the pDC, which is a high producer of IFN-alpha. However, transcriptional data show that IFN-beta (a type I IFN) and/or IFN-gamma (type II IFN) transcript levels correlate most highly with the IFN response that is seen in DM skin (Wong et al. 2012), and DM skin has elevated levels of IFN-gamma protein (Caproni et al. 2004).

Mechanism of KC Death

The exact cause of KC injury in DM skin is unknown. Some investigators have reported close apposition of both CD8$^+$ T cells and pDC to the dying KC, suggesting a role for these two cell types (Magro et al. 2010). However, granzyme B (GrB) expression is reported to be very low in DM skin, and so, it is possible that other modes of cytotoxicity are involved (Grassi et al. 2009). Recent data suggest that the Fas/FasL mechanism might be operative (as opposed to a GrB-mediated event) (Torchia et al. 2010). Other studies suggest that KC death may be a p53-mediated apoptosis event that is associated with decreased levels of Bcl2 (Pablos et al. 1999; Dourmishev and Wollina 2006).

Vascular Injury

As in muscle, DM skin shows signs of vasculopathy. This is apparent both clinically (periungual telangiectasias, and cutaneous ulcers) and microscopically (endothelial cell swelling, ectasia, and fibrin deposition)(Crowson and Magro 1996). Although there is demonstrated pathologic rarefication of superficial vessels in DM (Crowson and Magro 1996), interestingly, there is actually clinically a gross increase in blood flow to DM skin (Dawn et al. 2007). Vasculopathy may be more pronounced in

certain DM subtypes, as patients who have antibodies to MDA5 demonstrate a clearly higher risk of cutaneous ulcers characterized by vessel damage and inflammation (Fiorentino et al. 2011; Chaisson et al. 2012). The mechanism of this vessel injury is unclear, although it is known that IFN (and its induced gene products) can induce endothelial cell injury. A recent study suggested that vasculopathy was associated with high levels of MxA expression in the endothelial cells (Magro et al. 2010). As in the muscle, DM skin is characterized by deposition of the membrane attack complex, C3d and C4d, in the vessel walls, suggesting a role for complement-mediated damage (Mascaro et al. 1995; Magro and Crowson 1997). It may be that target antigens are actually expressed in the vasculature and direct an adaptive immune response (and/or complement-mediated damage) to the vessel, but how this would relate to KC damage seen in DM is not known.

Chondroitin sulfate core protein serglycin, a known regulator of leukocyte adhesion, is significantly overexpressed in DM but not in lupus endothelial cells, and may play a role in vascular damage. Additionally, recent evidence suggests a role for angiopoietin-like protein 2 (angptl2). Angpl2 is a secreted glycoprotein with homology to the angiopoietins and may play a role in endothelial modification and inflammation through activation of NF-κB (Kim and Werth 2011). Transgenic mice expressing Angptl2 driven by a keratinocyte-specific promoter K14 (K14-Angpl2) have phenotypic and histologic similarity to DM, exhibiting heliotrope periocular rash, body rash, and increased dermal mucin (Ogata et al. 2012).

Environmental Triggers

It is postulated that DM skin disease is the result of a specific genetic interaction with an environmental response (Robinson and Reed 2011). Ultraviolet light is clearly one trigger in DM skin – DM patients have a lower threshold for UV-induced erythema (Dourmishev et al. 2004), and DM (especially anti-Mi2 positive disease) is more prevalent in areas with a higher UV index (Okada et al. 2003; Love et al. 2009). One theory is that UV induces KC injury which then results in cytokines that can activate the innate immune system and/or cause overexpression, modification, or redistribution of putative antigens, resulting in an immune response. Interestingly, UV radiation has been shown to stabilize (and thus increase epidermal levels) of the Mi2 protein, which might be a mechanism for perpetuation or initiation of an antigens-specific immune response in the skin (Burd et al. 2008).

Physical factors such as stretch have also been implicated in DM, as the inflammation is often centered over the extensor surface of joints. Werth and colleagues have shown that the glycosaminoglycans in dermal skin of DM patients contain large amounts of chondroitin-4-sulfate (C4S), a molecule with both pro- and anti-inflammatory effects (Kim and Werth 2011). Interestingly, Gottron papule lesions contain a larger relative amount of C4S than other areas, as well as increased levels of CD44v7 (a ligand for C4S) and its binding partner, osteopontin (OPN) (Kim et al. 2012). The authors show that mechanical stretch induces expression of CD44v7, and may explain localization of rash to overlying joints. They provide evidence that binding of OPN to CD44v7 induces monocyte adhesion and may provide a feed-forward mechanism for inflammation in areas of high stretch.

Cross-References

- ▶ Cancer and Dermatomyositis
- ▶ Myositis, Pathogenesis
- ▶ Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies

References

Antiga E, Kretz CC, Klembt R, et al. Characterization of regulatory T cells in patients with dermatomyositis. J Autoimmun. 2010;35(4):342–50.

Burd CJ, Kinyamu HK, Miller FW, Archer TK. UV radiation regulates Mi-2 through protein translation and stability. J Biol Chem. 2008;283(50):34976–82.

Caproni M, Torchia D, Cardinali C, et al. Infiltrating cells, related cytokines and chemokine receptors in lesional skin of patients with dermatomyositis. Br J Dermatol. 2004;151(4):784–91.

Chaisson NF, Paik J, Orbai AM, et al. A novel dermatopulmonary syndrome associated with MDA-5 antibodies: report of 2 cases and review of the literature. Medicine. 2012;91(4):220–8.

Crowson AN, Magro CM. The role of microvascular injury in the pathogenesis of cutaneous lesions of dermatomyositis. Hum Pathol. 1996;27(1):15–9.

Dawn A, Thevarajah S, Cayce KA, et al. Cutaneous blood flow in dermatomyositis and its association with disease severity. Skin Res Technol. 2007;13(3):285–92.

Dourmishev LA, Wollina U. Dermatomyositis: immunopathologic study of skin lesions. Acta Dermatovenerol Alp Panonica Adriat. 2006;15(1):45–51.

Dourmishev L, Meffert H, Piazena H. Dermatomyositis: comparative studies of cutaneous photosensitivity in lupus erythematosus and normal subjects. Photodermatol Photoimmunol Photomed. 2004; 20(5):230–4.

Fiorentino D, Chung L, Zwerner J, et al. The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to MDA5 (CADM-140): a retrospective study. J Am Acad Dermatol. 2011;65(1):25–34.

Grassi M, Capello F, Bertolino L, et al. Identification of granzyme B-expressing CD-8-positive T cells in lymphocytic inflammatory infiltrate in cutaneous lupus erythematosus and in dermatomyositis. Clin Exp Dermatol. 2009;34(8):910–4.

Hausmann G, Mascaro Jr JM, Herrero C, et al. Cell adhesion molecule expression in cutaneous lesions of dermatomyositis. Acta Derm Venereol. 1996; 76(3):222–5.

Kashiwagi M, Morgan BA, Georgopoulos K. The chromatin remodeler Mi-2beta is required for establishment of the basal epidermis and normal differentiation of its progeny. Development. 2007;134(8):1571–82.

Kim JS, Werth VP. Identification of specific chondroitin sulfate species in cutaneous autoimmune disease. J Histochem Cytochem. 2011;59(8):780–90.

Kim JS, Bashir MM, Werth VP. Gottron's Papules exhibit dermal accumulation of CD44 variant 7 (CD44v7) and its binding partner osteopontin: a unique molecular signature. J Invest Dermatol. 2012;132(7):1825–32.

Love LA, Weinberg CR, McConnaughey DR, et al. Ultraviolet radiation intensity predicts the relative distribution of dermatomyositis and anti-Mi-2 autoantibodies in women. Arthritis Rheum. 2009;60(8):2499–504.

Magro CM, Crowson AN. The immunofluorescent profile of dermatomyositis: a comparative study with lupus erythematosus. J Cutan Pathol. 1997;24(9):543–52.

Magro CM, Segal JP, Crowson AN, Chadwick P. The phenotypic profile of dermatomyositis and lupus erythematosus: a comparative analysis. J Cutan Pathol. 2010;37(6):659–71.

Mascaro Jr JM, Hausmann G, Herrero C, et al. Membrane attack complex deposits in cutaneous lesions of dermatomyositis. Arch Dermatol. 1995;131(12):1386–92.

McNiff JM, Kaplan DH. Plasmacytoid dendritic cells are present in cutaneous dermatomyositis lesions in a pattern distinct from lupus erythematosus. J Cutan Pathol. 2008;35(5):452–6.

Ogata A, Endo M, Aoi J, et al. The role of angiopoietin-like protein 2 in pathogenesis of dermatomyositis. Biochem Biophys Res Commun. 2012;418(3):494–9.

Okada S, Weatherhead E, Targoff IN, et al. Global surface ultraviolet radiation intensity may modulate the clinical and immunologic expression of autoimmune muscle disease. Arthritis Rheum. 2003;48(8):2285–93.

Pablos JL, Santiago B, Galindo M, et al. Keratinocyte apoptosis and p53 expression in cutaneous lupus and dermatomyositis. J Pathol. 1999;188(1):63–8.

Robinson AB, Reed AM. Clinical features, pathogenesis and treatment of juvenile and adult dermatomyositis. Nat Rev Rheumatol. 2011;7(11):664–75.

Shrestha S, Wershil B, Sarwark JF, et al. Lesional and nonlesional skin from patients with untreated juvenile dermatomyositis displays increased numbers of mast cells and mature plasmacytoid dendritic cells. Arthritis Rheum. 2010;62(9):2813–22.

Smith ES, Hallman JR, DeLuca AM, et al. Dermatomyositis: a clinicopathological study of 40 patients. Am J Dermatopathol. 2009;31(1):61–7.

Solomon GJ, Magro CM. Foxp3 expression in cutaneous T-cell lymphocytic infiltrates. J Cutan Pathol. 2008;35(11):1032–9.

Torchia D, Caproni M, Volpi W, et al. The Fas/Fas ligand system, rather than granzyme B, may represent the main mediator of epidermal apoptosis in dermatomyositis. Clin Exp Dermatol. 2010;35(6):669–70.

Wenzel J, Tuting T. An IFN-associated cytotoxic cellular immune response against viral, self-, or tumor antigens is a common pathogenetic feature in "interface dermatitis". J Invest Dermatol. 2008; 128(10):2392–402.

Wenzel J, Scheler M, Bieber T, Tuting T. Evidence for a role of type I interferons in the pathogenesis of dermatomyositis. Br J Dermatol. 2005;153(2):462–3; author reply 3–4.

Wenzel J, Schmidt R, Proelss J, et al. Type I interferon-associated skin recruitment of CXCR3+ lymphocytes in dermatomyositis. Clin Exp Dermatol. 2006;31(4):576–82.

Wong D, Kea B, Pesich R, et al. Interferon and biologic signatures in dermatomyositis skin: specificity and heterogeneity across diseases. PLoS One. 2012;7(1): e29161.

Zahn S, Barchet W, Rehkamper C, et al. Enhanced skin expression of melanoma differentiation-associated gene 5 (MDA5) in dermatomyositis and related autoimmune diseases. J Am Acad Dermatol. 2011;64(5):988–9.

Discoid Lupus

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Synonyms

Chronic cutaneous lupus; Cutaneous only lupus; Skin lupus

Definition

Discoid lupus (DLE) is a chronic, scarring skin disease characterized by clinical, histologic, and immunopathologic findings, commonly on the face over the cheeks and bridge of the nose in a "malar" or "butterfly" distribution.

Skin disease is the second most common clinical manifestation of lupus, after joint inflammation, and is divided into acute, subacute, and chronic cutaneous forms. Of the chronic cutaneous forms, discoid lupus is the most characteristic with prominence on the face, ears, and scalp.

These "discoid" or coin-shaped lesions frequently progress to permanent scars with significant effect on the quality of life in those individuals with the disease, suggesting that prevention as well as early and efficient management towards remission be the goal of the treating physician. There are variants of DLE including the hypertrophic or lichen planus overlap form as well as the palmar/plantar or acral type.

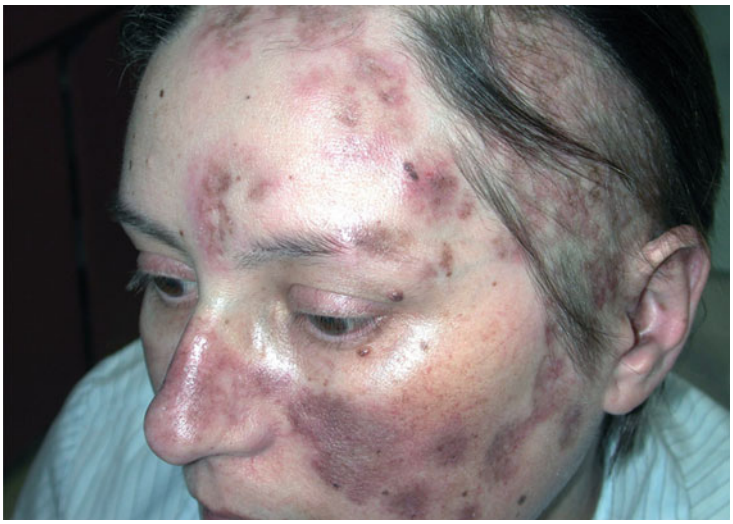
Contrary to SLE, with a female to male ratio of at least 8:1, chronic cutaneous lupus including DLE is also common in men with a female to male ratio of 6:5. The increased frequency of SLE among women has been attributed to an estrogen hormonal effect, which does not appear to play a significant role in cutaneous-only disease. This is often underrecognized and many men will not seek medical attention because of the stigma that this is a "female" disease.

There is a significant photosensitive and photoinduction component in the majority of patients with varied action spectrums. UVB is the classic action spectrum for induction of DLE, but UVA also plays a role, explaining why patients are sometimes frustrated when the standard sunscreens with limited UVA protection do not work. In addition, because UVA is present from dawn to dusk and travels through window glass, patients may be unaware of exposure. Also, the time frame between exposure and onset of lesions may be prolonged. Koebner phenomenon (Berger et al. 2012) to localized trauma does occur and also relates to thermal injury. Recently, the role of smoking as well as compliance to medications has been explored with regard to resistance to treatment.

Patients with DLE usually do not fulfill four or more of the criteria used to classify SLE and serologic abnormalities are not common (Vasquez et al. 2012). However, since DLE may occur in patients with SLE and some patients (<5 %) with DLE progress to SLE, a positive ANA when done by the IFA method may be identified and when of moderate to high titer may indicate the potential for extracutaneous disease (Chong et al. 2012). The "protective" effect of DLE regarding severe renal disease was recently reviewed (Merola et al. 2011).

It is unclear whether DLE lesions are representative of the pathologic dysfunction seen in SLE in other organ systems (Lin et al. 2007). Numerous studies have focused on the role of IL-17-producing Th17 cells in SLE. Elevated IL-17 levels and increased numbers of Th17 cells in the serum/blood of SLE patients have been reported. However, recent evidence suggests that Th1, rather than Th17, cells are the predominant T cell population in DLE lesions (Jabbari et al. 2014). TNF-alpha is also increased in lesional skin of DLE patients, and stimulation of TNF-alpha leads to increased production of IFN-gamma, whose aberrant expression is associated with a number of autoimmune disorders including SLE (Nabatian et al. 2012).

Multiple susceptibility genes play a role and several candidate genes previously associated with lupus have been confirmed (Braunstein et al. 2012),



Discoid Lupus, Fig. 1 Discoid lupus erythematosus (DLE) with chronic scarring alopecia

additional lupus-associated genes and gene loci have been identified, and variants in a novel gene (ITGAM) have been explored (Järvinen et al. 2010).

The clinical features (Fig. 1) are best described as erythematosus and scaly with central atrophy associated with loss of pigment and an inflamed, darker border with follicular plugging and scale-crust. With time the lesions leave post-inflammatory pigmentary (PIH) changes and atrophy with loss of skin markings and epidermal thinning. When on the scalp, permanent hair loss (scarring alopecia) occurs and may become widespread. The lips may become involved and when long-standing an increased incidence of squamous cell carcinoma may occur (scar carcinoma). The acronym *PASTE* is useful in initial diagnosis of active lesions as follows:

P: follicular plugging
A: atrophy
S: scale
T: telangiectasias
E: erythema

The histologic features characteristically show a superficial and deep lymphocytic infiltrate in proximity to the hair follicle and other adnexal structures, a prominent PAS + staining of a thickened basement membrane, as well as vacuolar alteration of the basal cells at the interface (interface dermatitis). Mucin may be present as well within the dermis. Direct immunofluorescence of an active lesion (lupus band test of lesional skin) usually reveals positive staining of IgG at the basement membrane in a "lumpy-bumpy" pattern. IgM deposition may occur in concert with IgG but when found alone is considered less specific. Lupus band testing of non-lesional, non-light-exposed skin has been utilized as a predictor of systemic, particularly renal, disease.

Lesions tend to occur on areas of photo-exposure such as the malar area of the face and nose but with a notable sparing of the nasolabial fold. This is in contradistinction to the malar rash of dermatomyositis which targets this area. In addition, rosacea also targets the nasolabial fold and is found in association with varying degrees of pustular lesions. When the scalp is involved, there is concordance with lesions of the ear. The "V of the neck" is also a prominent area of involvement. If DLE extends below the neck and is more disseminated (generalized DLE), it is considered a "marker of transition" as the incidence of systemic disease is increased.

Treatment

The avoidance of ultraviolet exposure in both the UVB and UVA range is important in the prevention of recurrence. Patients need to be educated

with regard to sources of exposure including artificial sources such as exposed fluorescent fixtures. UVA protection efficacy from sunscreen is not uniformly available and does not relate to SPF UVB labeling. Photosensitizing foods, supplements, and medications need to be carefully reviewed. Smoking may be a factor in some patients and it is prudent to discontinue their use. Except for photosensitizing foods such as alfalfa, some artificial sweeteners, and lime, there is no specific diet that appears to have major influence on the course of the disease in the majority of patients.

Limited disease on the skin is usually treated with topical corticosteroids or calcineurin inhibitors. Fluocinonide 0.05 % cream and tacrolimus ointment 0.1 % are useful in many patients, whereas lower potency topical steroids are generally ineffective. If lesions effect the scalp, intralesional doses of triamcinolone in the range of 0.3–0.5 mg/cc may minimize permanent hair loss.

There are no medications specifically indicated for the treatment of CLE (Jessop et al. 2009). Many of the drugs utilized are approved for use in SLE, and hydroxychloroquine is an excellent example of this strategy. Most new drug studies for DLE are utilizing the CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index) as an outcome instrument for evaluation of cutaneous lupus (Albrecht et al. 2005).

Antimalarial drugs when used singly or in combination (hydroxychloroquine with quinacrine) will maintain remission in the majority of DLE patients encountered (Costedoat-Chalumeau et al. 2013). Dosing of hydroxychloroquine or chloroquine is based on mg/kg/day to minimize ocular toxicity. Both maximum daily dose and cumulative dose need to be considered. Hydroxychloroquine maximum dosing is generally 6.5 mg/kg/day, whereas chloroquine is 3.0 mg/kg/day. Quinacrine has no such guideline because of its relative sparing of retinal toxicity.

Acitretin (50 mg/day) has been shown to be efficacious in DLE although adverse effects are more frequent than with antimalarials. Other retinoids, such as isotretinoin, have also been utilized, but these agents are classified as second-line therapies for the treatment of CLE by the American Academy of Dermatology guidelines.

R-salbutamol, a B2 adrenergic receptor agonist commonly used in the treatment of asthma, was noted to inhibit transcription of inflammatory genes and the subsequent production of interleukin (IL)-2 and interferon (INF)-gamma. Studies suggest R-salbutamol may be an effective new topical therapy alternative for DLE (Jemec et al. 2009).

Various laser modalities have been utilized both to treat active disease (Erceg et al. 2009) as well as to repair lupus scarring in patients with DLE (Franks 2004). Guidelines are preliminary but have yet to be firmly established, but this technology appears promising, particularly with the intent to repair lupus-damaged skin.

Systemic corticosteroids are generally avoided in DLE patients due to cumulative side effects as well as their non-remittive nature. Short courses to prevent scarring or as "bridge therapy" until other agents become active are certainly warranted. Doses similar to that utilized in severe poison ivy or contact dermatitis are recommended.

Dapsone in doses of 25–100 mg/day has had beneficial effect in some patients who have not responded to or are intolerant to the antimalarials. Careful monitoring is required because of the hemolysis that invariably occurs at therapeutic levels. Because of its additional effect on neutrophil chemotaxis, some neutrophil-rich bullous forms of cutaneous disease also respond to the drug.

Thalidomide has been found to improve cutaneous lupus erythematosus in 90 % of patients utilizing doses between 50 and 400 mg daily (Cuadrado et al. 2005). It is not remittive, and rebound occurs in the majority of patients within 3–6 weeks after discontinuation. The neuropathic (Bastuji-Garin et al. 2002) and thrombophilic side effects (Piette et al. 2002) need to be individually addressed, and the use of double

contraception enforced. Those patients recalcitrant to the antimalarials are best considered for the addition of thalidomide to their regimen. Lenalidomide, an analogue of thalidomide, has recently been shown to improve patients with DLE. Similar side effects were noted.

Azathioprine in low doses, 25–100 mg/day, has been utilized for many years in patients with recalcitrant disease and has been a very useful adjunctive agent. Recently, methotrexate has been reintroduced as an excellent agent for recalcitrant disease in doses of up to 25 mg/week. Subcutaneous/intramuscular administration appears to minimize adverse side effects and may increase efficacy.

Mycophenolate mofetil and mycophenolate sodium have become increasingly popular, particularly because these drugs have become popular in lupus nephritis. They are well tolerated and have a wide therapeutic window; doses ranging from 500 mg/day to 2 g per day have been effective in DLE.

Apremilast, another analogue of thalidomide, is a novel oral PDE4 enzyme inhibitor capable of blocking leukocyte production of IL-12, IL-23, TNF-a, and INF-gamma with subsequent suppression of Th1- and Th17-mediated immune responses. It was recently shown to be effective in DLE recalcitrant to conventional therapy, with minimal side effects (DeSouza et al. 2012).

Rituximab, an anti-CD20 chimeric, monoclonal antibody, originally utilized only in B cell lymphoma, has gained acceptance for a number of autoimmune diseases including rheumatoid arthritis and granulomatosis with polyangiitis (Wegener's). Its SLE off-label autoimmune dosing is 375 mg/m^2 × 2 doses, 2 weeks apart. Infusion-related side effects are frequent and onset of clinical response may be delayed. There are significant concerns about progressive multifocal leukoencephalopathy (PML) infection in SLE patients.

Belimumab is a monoclonal antibody that inhibits soluble B-lymphocyte stimulator (anti-BlyS) and improves SLE disease activity. In a recent study, 42 SLE patients were on Belimumab intravenously for at least 3 months. Of those, 23 (55 %) patients clinically responded by 3 months with marked improvement in arthritis and/or rash, including those patients with SLE who also had DLE lesions (Akanase et al. 2013). It will shortly be available by the subcutaneous route.

The TNF-alpha inhibitors are controversial because of the systemic upregulation of autoantibody production leading to induction or acceleration of systemic disease tocilizumab prevents the binding of IL-6 to membrane-bound and soluble IL-6 receptor. It is approved by the US Food and Drug Administration for rheumatoid arthritis and juvenile inflammatory arthritis refractory to other agents and was beneficial to the skin in a patient with leukocytoclastic vasculitis who also had tumid lupus, another form of chronic cutaneous disease (Makol et al. 2012). Ustekinumab is a human monoclonal antibody directed against interleukin-12 and interleukin-23 and has recently been shown to be effective in SCLE and DLE (DeSouza et al. 2012).

Cross-References

- ▶ Hair Loss in Lupus Erythematosus
- ▶ Immunology of Alopecia in Autoimmune Skin Disease

References

Akanase A, Reddy A, Buyon JP, Franks AG Jr, Furie RA, Kamen DL, Manzi S, Petri M, Ramsey-Goldman R, Tseng C-E, van Vollenhoven RF, Wallace DJ. Favorable Clinical Response to Belimumab at Three Months. Arthritis & Rheumatism. 2013;65(S10):S668–S668.

Albrecht J, Taylor L, Berlin JA, Dulay S, Ang G, Fakharzadeh S, Kantor J, Kim E, Militello G, McGinnis K, Richardson S, Treat J, Vittorio C, Van Voorhees A, Werth VP. The CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index): an outcome instrument for cutaneous lupus erythematosus. J Invest Dermatol. 2005;125(5):889–94.

Bastuji-Garin S, Ochonisky S, Bouche P, Gherardi RK, Duguet C, Djerradine Z, Poli F, Djerradine J, Thalidomide Neuropathy Study Group. Incidence and risk factors for thalidomide neuropathy: a prospective study of 135 dermatologic patients. J Invest Dermatol. 2002;119(5):1020–6.

Berger E, Robinson M, Patel R, Franks Jr AG. Koebner phenomenon to heat in cutaneous (discoid) lupus

erythematosus (lupus ab-igne). Dermatol Online J. 2012;18(12):17.
Braunstein I, Klein R, Okawa J, Werth VP. The interferon-regulated gene signature is elevated in subacute cutaneous lupus erythematosus and discoid lupus erythematosus and correlates with the cutaneous lupus area and severity index score. Br J Dermatol. 2012;166(5):971–5.
Chong BF, Song J, Olsen NJ. Determining risk factors for developing systemic lupus erythematosus in patients with discoid lupus erythematosus. Br J Dermatol. 2012;166(1):29–35.
Costedoat-Chalumeau N, Galicier L, Aumaître O, Francès C, Le Guern V, Lioté F, Smail A, Limal N, Perard L, Desmurs-Clavel H, Boutin DL, Asli B, Kahn JE, Pourrat J, Sailler L, Ackermann F, Papo T, Sacré K, Fain O, Stirnemann J, Cacoub P, Jallouli M, Leroux G, Cohen-Bittan J, Tanguy ML, Hulot JS, Lechat P, Musset L, Amoura Z, Piette JC; on behalf of Group PLUS. Hydroxychloroquine in systemic lupus erythematosus: results of a French multicenter controlled trial (PLUS Study)controlled trial (PLUS) Ann Rheum Dis. 2013;72(11):1786–92.
Cuadrado MJ, Karim Y, Sanna G, Smith E, Khamashta MA, Hughes GR. Thalidomide for the treatment of resistant cutaneous lupus: efficacy and safety of different therapeutic regimens. Am J Med. 2005;118(3):246–50.
DeSouza A, Strober BE, Merola JF, Oliver S, Franks Jr AG. Apremilast for discoid lupus erythematosus: results of a phase 2, open-label, single-arm, pilot study. J Drugs Dermatol. 2012;11(10):1224–6.
De Souza A, Ali-Shaw T, Strober BE, Franks Jr AG. Successful treatment of subacute lupus erythematosus with ustekinumab. Arch Dermatol. 2011;147(8):896–8.
Erceg A, Bovenschen HJ, van de Kerkhof PC, et al. Efficacy and safety of pulsed dye laser treatment for cutaneous discoid lupus erythematosus. J Am Acad Dermatol. 2009;60:626–32.
Franks AG. Ask the experts: is the use of laser safe for people with lupus. Lupus Now. 2004;FALL:32–3.
Jabbari A, Suarez-Farinas M, Fuentes-Duculan J, Gonzalez J, Cueto I, Franks AG Jr, Krueger JG. J Invest Dermatol. 2014;134:87–95.
Järvinen TM, Hellquist A, Koskenmies S, Einarsdottir E, Panelius J, Hasan T, Julkunen H, Padyukov L, Kvarnström M, Wahren-Herlenius M, Nyberg F, D'Amato M, Kere J, Saarialho-Kere U. Polymorphisms of the ITGAM gene confer higher risk of discoid cutaneous than of systemic lupus erythematosus. PLoS One. 2010;2:5(12).
Jemec GB, Ullman S, Goodfield M, et al. A randomized controlled trial of R-salbutamol for topical treatment of discoid lupus erythematosus. Br J Dermatol. 2009;161:1365–70.
Jessop S, Whitelaw DA, Delamere FM. Drugs for discoid lupus erythematosus. Cochrane Database Syst Rev. 2009;7(4):CD002954.
Lin JH, Dutz JP, Sontheimer RD, Werth VP. Pathophysiology of cutaneous lupus erythematosus. Clin Rev Allergy Immunol. 2007;33:85–106.
Makol A, Gibson LE, Michet CJ. Successful use of interleukin 6 antagonist tocilizumab in a patient with refractory cutaneous lupus and urticarial vasculitis. J Clin Rheumatol. 2012;18:92–5.
Merola JF, Chang CA, Sanchez MR, Prystowsky SD. Is chronic cutaneous discoid lupus protective against severe renal disease in patients with systemic lupus erythematosus? J Drugs Dermatol. 2011;10(12):1413–20.
Nabatian et al. Tumor necrosis factor a release in peripheral blood mononuclear cells of cutaneous lupus and dermatomyositis patients. Arthritis Res Ther. 2012;14(1):R1.
Piette JC, Sbai A, Francès C. Warning: thalidomide-related thrombotic risk potentially concerns patients with lupus. Lupus. 2002;11(2):67–70.
Vasquez R, Tseng LC, Victor S, Zhang S, Chong BF. Autoantibody and clinical profiles in patients with discoid lupus and borderline systemic lupus. Arch Dermatol. 2012;148(5):651–5.

Discoid SLE

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Synonyms

Chronic cutaneous lupus erythematosus; Discoid lupus erythematosus; Lupus erythematosus chronicus discoides

Definition

Discoid lupus erythematosus (DLE) is a chronic, autoimmune, inflammatory disease of the skin characterized by coin-shaped (discoid) lesions that give rise to scarring, atrophy, alopecia, and pigment changes as they heal. These lesions often occur on sun-exposed areas such as the face, ears, and hands although any cutaneous region as well as some mucosa can be affected.

Classification

The disease can present as localized, generalized, or childhood DLE (William et al. 2011)

1. Localized DLE: Lesions are found above the neck, with favored sites including the malar areas, bridge of nose, scalp (leading to alopecia), ears, and lower lip. This form of DLE may rarely involve the mucosa of the mouth, nose, eye, or vulva.
2. Generalized DLE: Less common than the localized form, generalized DLE often affects the thorax and upper extremities in addition to the head and neck. All degrees of severity may be encountered with this form.
3. Childhood DLE: Clinical presentation and disease course in children with DLE is similar to that observed in adults. However, there is a lower frequency of photosensitivity and a higher rate of transition to systemic lupus erythematosus (SLE). In addition, the childhood form does not demonstrate female predominance.

Epidemiology

Individuals with a family history of DLE or other autoimmune conditions are at an increased risk of acquiring the disease (Lawrence et al. 1987). Female predominance is observed in DLE, with a 2:1 female to male ratio. It may occur at any age, but most commonly affects individuals between 20 and 40 years of age. According to studies in the United States, DLE is more prevalent in African-American populations than in other ethnic groups (Molina et al. 1997). Other risk factors for developing DLE include exposure to ultraviolet (UV) light and cigarette smoking (Miot et al. 2005).

Etiopathogenesis

The etiology of DLE is incompletely understood, but it is generally believed to be caused by a genetic predisposition triggered by environmental factors. In DLE, a dysfunctional immune system produces autoantibodies that attack healthy skin tissue, resulting in inflammation and eruption of a rash.

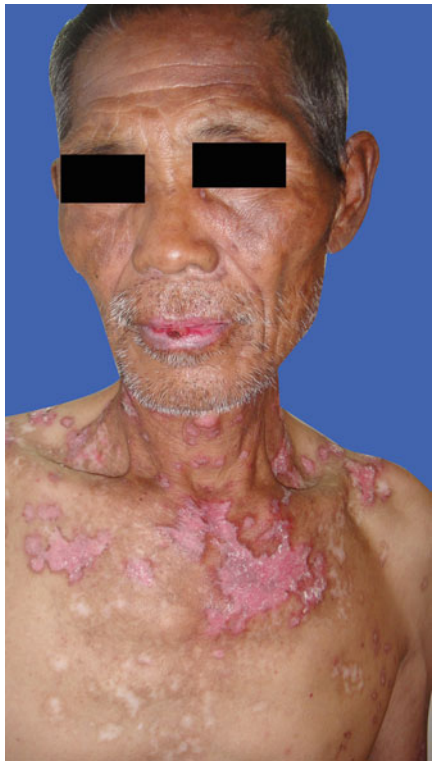
Genetic factors: Members of the human major histocompatibility complex gene family have been implicated in the susceptibility and development of DLE (López-Tello et al. 2007).

Environmental factors: UV radiation is known to stimulate the expression of nuclear and cytoplasmic antigens like Ro/SSA on the surface of keratinocytes. These antigens are then targeted by circulating anti-Ro/SSA antibodies, leading to antibody-dependent cellular cytotoxicity and ultimately skin damage (LeFeber et al. 1984; Norris et al. 1997). UV irradiation can also upregulate Gadd45a expression and induce DNA methylation, and UV light can trigger lupus flares (Li et al. 2010). Smoking is also highly associated with the development of DLE, as it activates autoimmune lymphocytic activity, thus promoting inflammation. In addition, hormonal factors such as long-term exposure to estrogen can also lead to the development of DLE (Meier et al. 1998), which helps explain the higher prevalence of the disease in women. Drugs such as voriconazole, fluorouracil, and certain NSAIDs have been reported to induce DLE in individuals with predisposing genetic factors (Denning and Griffiths 2001).

Clinical Features

Classic DLE lesions are characterized by their red, raised, and coin-shaped (discoid) appearance. Lesions tend to start healing from the center with atrophy, scarring, hypopigmentation, and telangiectasia commonly occurring on sun-exposed areas of the face and scalp (Fig. 1).

Initial lesions consist of erythematous macules or plaques that later develop into follicular scales and crusts. There is little or no associated pain or pruritus. The lesions gradually expand, producing areas of peripheral inflammation and hyperpigmentation, leaving a central region of scarring with hypopigmentation and telangiectasia. Scalp lesions may lead to changes in hair pigment and even permanent alopecia if scarring occurs due to delayed treatment.



Discoid SLE, Fig. 1 A 68-year-old Chinese man presenting with generalized discoid lupus erythematosus involving the face and thorax. Typical lesions appear red, raised, and coin shaped, with central atrophy and scarring. Large, atypically shaped lesions result from merging of the smaller, typical discoid lesions. Mucosal lesions affecting the lips are also evident. The healed lesions have given rise to hypopigmented areas (e.g., lower chest), as well as hyperpigmentation and scarring (e.g., face) (Author took the picture himself with the permission of the patient in the Outpatient Department of Second Xiangya Hospital of Central South University)

In darker skinned individuals, late lesions appear hyperpigmented or depigmented while in lighter skinned individuals, the plaques may appear gray or exhibit little pigment change. In general, lesions leave behind scars or discolored patches after they heal.

Mucosal lesions are also common. The most frequently affected site is the mouth, predominantly on the inner cheeks and lips. They are also encountered at other sites such as the vulva (Rowell and Goodfield 1998) and nasal mucosa.

About 5–10 % of DLE patients develop SLE (Healy et al. 1995), with generalized DLE being more likely to progress into SLE than the localized type. Squamous cell carcinoma may arise in long-standing lesions of DLE.

Histopathology

Biopsy and histopathological examination are done to confirm the diagnosis of suspected DLE. Characteristic histologic findings include hyperkeratosis and follicular plugging. There is vacuolar degeneration of the epidermal basal layer and follicular epithelium, resulting in pigment incontinence. Epidermal atrophy is usually present. A patchy perivascular and periadnexal infiltrate composed mainly of lymphocytes is seen in the superficial and deep dermis. Other notable findings include thickening of the basement membrane and increased mucin deposition in the dermis (Braun-Falco et al. 2000).

The above-mentioned histologic findings are typical of and consistent with active erythematous lesions. Chronic inactive lesions are atrophic with postinflammatory pigmentation and scarring observed throughout the dermis. Inactive lesions also display damaged pilosebaceous units and have little or no inflammatory infiltrate.

Direct immunofluorescence on skin tissue from lesions that have been active for several months will be positive in about 90 % of patients. There is a granular deposition of immunoglobulin and complement at the dermoepidermal junction. Skin biopsy should always be done on well-established, active lesions.

Diagnosis

Taking a complete medical history and doing a physical examination are a fundamental first step in establishing the diagnosis of DLE (Trozak et al. 2006). The former should include a smoking history, excessive UV exposure, drug history, family history of a similar disease, and a history of exposure to estrogen-containing contraceptives, among other things. The patient should give a detailed description of the initial occurrence and evolution of the skin lesions. After

taking the history, the lesions should be examined, focusing on recognizing the typical clinical features of DLE. A biopsy is then taken in order to confirm the diagnosis. Serological tests should also be done to rule out or detect progression to SLE. The presence of antibodies such as Antinuclear Antibodies (ANAs) in high titer and/or other multi-organ system signs and symptoms are highly suggestive of lesions being a result of SLE rather than DLE. Full blood count (FBC), erythrocyte sedimentation rate (ESR), and kidney function tests should also be part of the initial work-up to exclude any systemic involvement associated with SLE. Microscopy and culture of lesion scrapings may be necessary to rule out bacterial and fungal infections.

Differential Diagnosis

Some of the main differential diagnoses for DLE include SLE, subacute cutaneous lupus erythematosus (SCLE), plaque psoriasis, rosacea, polymorphous light eruption (PMLE), actinic keratosis, and lichen planopilaris (Weber and Fritsch 2005).

SLE presents with a characteristic malar rash and affects multiple organs. Lab tests show high titers of ANA, anti-dsDNA antibodies, and anti-RNP antibodies. SCLE lesions have typical erythematous crusted margins that heal without scarring and usually spare the face, mainly affecting the upper torso, neck, and arms. Biopsy shows minimal or absent basement membrane thickening. Psoriatic lesions are papules and plaques that have a characteristic silvery white scale and are mostly seen on the scalp and extensor surfaces (knees, elbows) of the body. Psoriatic nail pitting is also a common finding. Skin biopsy findings include intra-epidermal spongiform pustules and neutrophilic microabscesses within the stratum corneum.

Rosacea is characterized by erythema and chronic flushing with other findings such as rhinophyma, rough skin, telangiectasia, and an acneiform eruption. It typically affects convex facial areas (forehead, nose, cheeks, eyelids) in fair skinned people. Skin biopsy reveals sebaceous hyperplasia, edema, telangiectasia, and a nonspecific granulomatous infiltrate. PMLE lesions develop within 24 h of sun exposure, are usually itchy, and express several clinical forms with associated urticaria. Skin biopsy shows edema in the upper dermis. Actinic keratoses are rougher, hyperkeratotic, and usually have no scales. The lesions are smaller, greater in number, and occur in older patients with evidence of chronic actinic damage on facial skin.

Lichen planopilaris is a form of lichen planus that destroys hair follicles (usually of the scalp), replacing them with scar tissue and causing permanent alopecia. Typical lichen planus lesions affecting the skin, nails, and buccal mucosa with characteristic Wickham's striae are usually present. Skin biopsy shows a band-like subepidermal infiltrate of lymphocytes involving the follicles between the infundibulum and the isthmus with sparing of the lower portion.

Laboratory Tests

In addition to a biopsy, there are other tests that can be done to establish a diagnosis of DLE. ANA are positive in a minority of patients (less than 20 %) and their presence correlates with a more active and progressive clinical disease (Callen et al. 1985). Anti-dsDNA antibodies are positive in less than 5 % of patients and may correlate with increased disease severity and a poor prognosis. Anti-Ro/SSA antibodies are positive in 1–3 % of patients.

Erythrocyte sedimentation rate (ESR) may be slightly elevated or normal, while complement levels may be low or normal.

Treatment

General measures: Some general measures are essential in the successful management of DLE. First of all, patients should avoid exposure to sunlight and use a high sun-protection factor (SPF) sunscreen daily. Patients should also avoid exposure to excessive heat, cold, or trauma. Those with a history of smoking should be

strongly encouraged to quit. All of the above-mentioned factors have been found to either induce or exacerbate DLE. In addition, maintaining a healthy diet low in red meat and dairy products and high in omega-3 fatty acids (e.g., salmon, sardines) is thought to decrease inflammation and improve overall disease status. Vitamin A supplements may also enhance the resolution of skin lesions.

Topical treatment: Potent or superpotent topical steroids are the mainstay of DLE therapy and should be applied at least twice a day. Occlusion may improve the efficiency of topical steroids and reduce treatment duration. Prolonged treatment should be avoided to reduce the risk of side effects such as skin atrophy. Topical calcineurin inhibitors like tacrolimus can also be used as second-line topical therapy.

Intralesional steroids: Intralesional triamcinolone acetonide injections can be given if lesions do not respond to topical therapy. The steroid is injected into the lesions at intervals of 4–6 weeks. The minimum possible dose should be used. Side effects include cutaneous atrophy and dyspigmentation.

Systemic treatment: Antimalarials are the first-line agents in the systemic treatment of DLE. The most commonly used preparations are hydroxychloroquine, chloroquine, and mepacrine, with hydroxychloroquine being the first line. However, high doses or prolonged treatment with hydroxychloroquine may cause adverse effects such as retinal toxicity. Patients and physicians should therefore be on the lookout for any potential side effects. Patients should be informed that it may take more than 4 weeks to note any clinical improvement. Mepacrine may be added to either hydroxychloroquine or chloroquine to improve efficacy and prevent some of the major side effects (Von Schmiedeberg et al. 2000).

Thalidomide is one of the most useful therapeutic alternatives to antimalarials, although its use has been limited due to the associated risk of teratogenicity and polyneuropathy. It is very effective and generally well tolerated if administered in low doses.

Systemic corticosteroids may be used to treat DLE, though only for a short period of time (usually less than 3 weeks) due to their long-term side effects. However, they may be added to other regimens in patients with progressive or disseminated disease, or in localized disease that does not respond to other modes of therapy. Systemic corticosteroids can also be used for initial control of severe DLE while antimalarial therapy is being initiated.

Immunosuppressive agents such as cyclosporine, methotrexate, azathioprine, and mycophenolate mofetil have been employed as steroid-sparing agents in the management of refractory DLE. Several studies have found these agents to be very effective but close monitoring is essential due to potentially severe side effects.

Some additional treatments, including the use of acitretin, efalizumab, or cryotherapy, have been found beneficial in the management of DLE.

Prognosis

The prognosis for patients with DLE is generally favorable. Nevertheless, early diagnosis and treatment are crucial to prevent the various poor outcomes of the disease. Patients who progress to SLE have a much poorer prognosis. In addition, patients with generalized DLE have a poorer prognosis than those with localized DLE., Patients with abnormal laboratory tests are also likely to have a poor outcome of disease (Millard and Rowell 1979).

Cross-References

- ▶ Environment and Autoimmunity
- ▶ Juvenile Diseases: SLE in Children
- ▶ Mixed Connective Tissue Disease (MCTD)
- ▶ Psoriasis
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Gender and Hormone Influences
- ▶ Systemic Lupus Erythematosus, Genetics
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Braun-Falco O, Plewig G, Wolff HH, Burgdorf WHC. Diseases of connective tissue. In: Bischoff W, editor. Dermatology. Berlin/Heidelberg/New York: Springer; 2000. p. 800–1.

Callen JP, Fowler JF, Kulick KB. Serologic and clinical features of patients with discoid lupus erythematosus: relationship of antibodies to single-stranded deoxyribonucleic acid and of other antinuclear antibody subsets to clinical manifestations. J Am Acad Dermatol. 1985;13(51):748–55.

Denning DW, Griffiths CE. Muco-cutaneous retinoid-effects and facial erythema related to the novel triazole antifungal agent voriconazole. Clin Exp Dermatol. 2001;26:648–53.

Healy E, Kieran E, Rogers S. Cutaneous lupus erythematosus – a study of clinical and laboratory prognostic factors in 65 patients. Ir J Med Sci. 1995;164:113–5.

Lawrence JS, Martins CL, Drake GL. A family survey of lupus erythematosus. 1. Heritability. J Rheumatol. 1987;14:913–21.

LeFeber WP, Norris DA, Ryan SR, Huff JC, Lee LA, et al. Ultraviolet light induces binding of antibodies to selected nuclear antigens on cultured human keratinocytes. J Clin Invest. 1984;74:1545–51.

Li Y, Zhao M, Yin H, Gao F, Wu X, Luo Y, Zhao S, Zhang X, Su Y, Hu N, Long H, Richardson B, Lu Q. Overexpression of the growth arrest and DNA damage-induced 45alpha gene contributes to autoimmunity by promoting DNA demethylation in lupus T cells. Arthritis Rheum. 2010;62(5):1438–47.

López-Tello A, Rodríguez-Carreón AA, Jurado F, Yamamoto-Furusho JK, Castillo-Vázquez M, Chávez-Muñoz C, et al. Association of HLA-DRB1*16 with chronic discoid lupus erythematosus in Mexican mestizo patients. Clin Exp Dermatol. 2007;32:435–8.

Meier CR, Sturkenboom MC, Cohen AS, Jick H. Postmenopausal estrogen replacement therapy and the risk of developing systemic lupus erythematosus or discoid lupus. J Rheumatol. 1998;25(8):1515–9.

Millard LG, Rowell NR. Abnormal laboratory test results and their relationship to prognosis in discoid lupus erythematosus. A long-term follow-up study of 92 patients. Arch Dermatol. 1979;115(9):1055–8.

Miot HA, Bartoli Miot LD, Haddad GR. Association between Discoid Lupus erythematosus and Cigarette Smoking. Dermatology. 2005;211:118–22.

Molina JF, et al. Ethnic differences in the clinical expression of systemic lupus erythematosus: a comparative study between African-Americans and Latin Americans. Lupus. 1997;6:63–7.

Norris DA, Whang K, David-Bajar K, Bennion SD. The influence of ultraviolet light on immunological cytotoxicity in the skin. Photochem Photobiol. 1997;65:636–46.

Rowell NR, Goodfield MJD. Connective tissue diseases. In: Champion RH, Burton JL, Burns DA, Breathnach SM, editors. Textbook of dermatology, vol. 3. 6th ed. Oxford: Blackwell Scientific; 1998. p. 2448.

Trozak DJ, Tennenhouse DJ, Russell JJ. Lupus erythematosus. In: Weisberg RB, editor. Dermatology skills for primary care. New Jersey: Humana Press; 2006. p. 176–81.

Von Schmiedeberg S, Rönnau AC, Schuppe HC, Specker C, Ruzicka T, Lehmann P. Combination of antimalarial drugs mepacrine and chloroquine in therapy refractory cutaneous lupus erythematosus. Hautarzt. 2000;51:82–5.

Weber F, Fritsch P. Clinical differential diagnosis of cutaneous lupus erythematosus. In: Kuhn A, Lehman P, Ruzicka T, editors. Cutaneous lupus erythematosus. Berlin/Heidelberg/New York: Springer; 2005. p. 147–159.

William DJ, Berger TG, Elston DM. Connective tissue diseases. In: Gabbedy R, Pinczewski S, editors. Andrews' diseases of the skin: clinical dermatology, 11th ed. Philadelphia: Elsevier Saunders; 2011. p. 155.

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Endothelial Cells and Inflammation

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Definition

Endothelial cells and inflammation: Endothelial cells line the lumen of blood and lymphatic vessels and form the interface between the vasculature and organs of the body. Endothelial activation encompasses a range of endothelial responses to inflammatory signals including changes in thromboresistance, altered vasomotor tone, and loss of barrier function. When activated, the endothelium quickly facilitates cellular trafficking. Leukocyte activation and transmigration is crucial for normal innate and adaptive immunity. The term endothelial dysfunction may be applied to states in which the endothelial cell phenotype poses a net liability to the host. The endothelial response to injury can result in vasoconstriction, vasodilatation, vascular leakage, and inflammation. Endothelial activation can transform the internal vascular surface from a nonadhesive barrier into one that recruits leukocytes, is procoagulant, and furthers the inflammatory process.

Introduction

Endothelial cells line all blood and lymphatic vessels and form the interface between the vasculature and all organs of the body. Far from inert, the endothelium forms a dynamic interface which regulates vascular tone and cellular and nutrient trafficking. It is responsible for maintaining the appropriate balance of procoagulant and anticoagulant activity. The endothelium interacts with circulating blood cells in a highly regulated manner and communicates with circulating cells based on cues received from the local environment.

Classically endothelial activation referred to the observation that endothelial cells demonstrated increased leukocyte adhesion in response to inflammatory mediators such as cytokines. The term *endothelial activation* now encompasses a range of endothelial responses to inflammatory signals. These reactions encompass a broad spectrum, including changes in thromboresistance, altered vasomotor tone, and loss of barrier function. The process of endothelial activation, inflammatory cell adhesion, and transendothelial migration requires a tightly regulated series of changes on the part of both inflammatory and endothelial cells. These changes include

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morphologic cytoskeletal remodeling to optimize inflammatory cell rolling and firm adhesion.

The endothelium is critical to maintaining vascular homeostasis. Since the endothelium provides the barrier between the vasculature and each organ, endothelial dysfunction can result in deleterious effects. It is important to recognize that physiologic activation of the endothelium might be appropriate in some circumstances of cellular injury (toxins, hypoxia, laceration), in order to promote wound healing. In contrast, if the endothelial cell response is overwhelming, out of proportion to the injury, or if the stressor is severe, the endothelial response may exacerbate the injury and prove detrimental to the host. The term *endothelial dysfunction* may be applied to states in which the endothelial cell phenotype poses a net liability to the host. The endothelial response to injury can result in vasoconstriction, vasodilatation, vascular leakage, and inflammation. Endothelial activation can transform the internal vascular surface from a nonadhesive barrier into one that recruits leukocytes, is procoagulant, and furthers the inflammatory process.

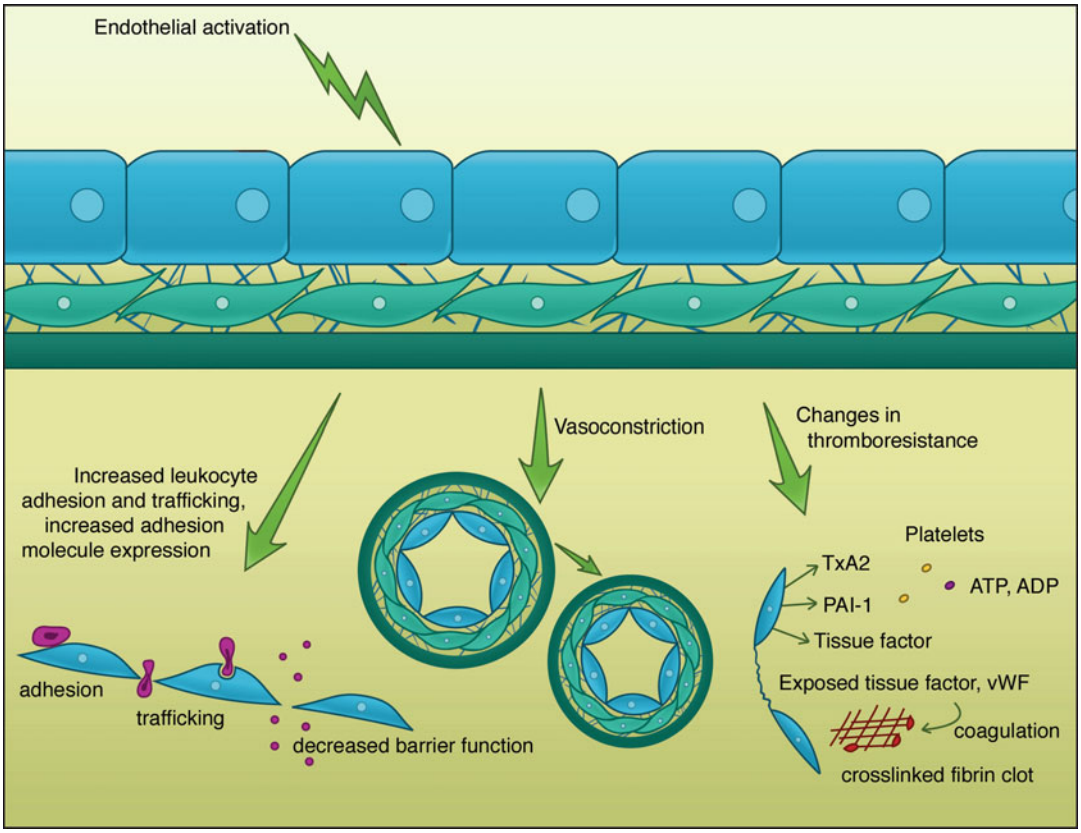
Endothelial activation can result from many stimuli. Examples include cytokine stimulation, toxins, trauma, antibodies, and ischemia. Ischemia, or the mismatch between blood supply and tissue demand, disrupts normal vascular homeostasis. Ischemia results in decreased pH, glucose, and oxygen supply. Further, ischemia hampers the tissue's ability to remove metabolic by-products. Decreased oxygen supply, or hypoxia, is common to many disease states, including pulmonary, cardiac, and peripheral arterial vascular disease. Over the course of evolution, the proximity of endothelial cells to the vascular lumen has resulted in a variety of *adaptive responses* of endothelial cells to disruptions in blood supply and composition. Undoubtedly the endothelial response to hypoxia evolved as a mechanism to prolong cellular survival despite oxygen deprivation. Among the oxygen-responsive transcription factors, early growth response protein-1 (Egr-1), nuclear factor κB (NFκB), and hypoxia-inducible factor-1α (HIF-1α) have proven to be important in mediating endothelial cell responses to hypoxia.

Endothelial activation in autoimmune disease is well described and likely contributes to the risk of early atherosclerosis in this population. Systemic inflammation, autoantibodies, and circulating immune complexes have been identified as important factors. Antiendothelial antibodies represent a heterogeneous group of autoantibodies directed against structural endothelial proteins. They are detectable in varied autoimmune and inflammatory conditions including interstitial lung disease, scleroderma, systemic lupus erythematosus (SLE), and systemic vasculitis (Narshi et al. 2011).

Barrier Function

In different vascular beds, endothelial cells take on characteristics which support the functions of the organ in which they reside. The vascular endothelium modulates the permeability of the blood vessel to solutes and fluid. In the brain, tight junctions between adjacent endothelial cells form the blood–brain barrier, vital to protecting the delicate parenchyma from toxins, metabolites, and pharmacological disturbances. In renal glomeruli, fenestrated endothelium permits filtration, whereas in the heart, non-fenestrated endothelium is present. Under basal conditions, non-fenestrated endothelium allows only restricted diffusion. *Restrictive diffusion* refers to the concept that the endothelium acts as a molecular sieve. Permeability of macromolecules is dependent upon their molecular radii as well as the particular barrier properties of the endothelium. Molecules can pass either by passive diffusion, restricted diffusion, or facilitated transport. If large gaps exist between cells (i.e., barrier function is lost), mass movement and Brownian motion are the forces which move solutes and molecules. The probability of solutes passing through the endothelium is inversely proportional to their Stokes radii. Restricted diffusion is key to maintaining protein gradients, while still allowing small solutes to move passively across the barrier via the paracellular route.

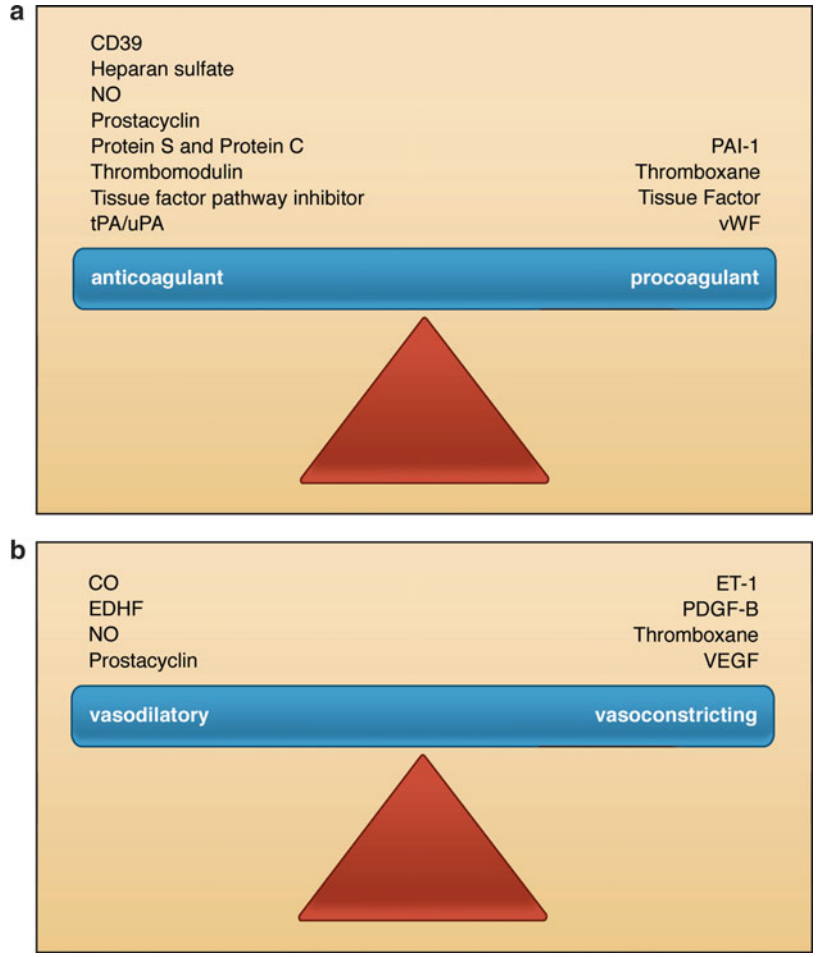
When activated, the endothelium quickly and proactively facilitates cellular trafficking (Fig. 1).



Endothelial Cells and Inflammation, Fig. 1 Endothelial cell activation results in diverse changes in the vascular environment. The endothelial cell response to injury, toxins, and inflammatory mediators includes increased leukocyte trafficking, changes in vasomotor tone, and decreased thromboresistance, to facilitate repair of injured tissue. When the endothelial cell response is disproportionate or poses a detriment to the host, this is termed endothelial dysfunction. The same endothelial cell responses may be considered adaptive or maladaptive, depending on the environmental trigger

Inflammatory mediators including thrombin, bradykinin, histamine, vascular endothelial growth factor (VEGF), interleukin (IL)-1, and tumor necrosis factor (TNF) disrupt cell-cell adhesion, causing intercellular gaps and thereby allowing unrestricted diffusion of solutes. Similarly, after exposure to hypoxia, activated endothelial cells retract, causing gaps between cells. This heralds the loss of endothelial barrier function and manifests as increased capillary leakage and interstitial edema. Solutes, proteins, and leukocytes may pass through the endothelium as diffusion becomes less restricted.

Interstitial edema as a result of increased capillary permeability after stroke contributes to infarct expansion. In septic shock, loss of barrier function and capillary leakage result in hypotension, decreased organ perfusion, organ dysfunction, and eventual death. Hypoxic stimulation of transcription factors HIF-1 and Egr-1 is known to upregulate VEGF, an inducer of increased vascular permeability (Ten and Pinsky 2002). Hypoxia-induced upregulation of Egr-1 has been shown to be detrimental in the setting of ischemic stress. For example, decreased pulmonary capillary leak and improved survival were noted after pulmonary ischemia/reperfusion in an animal model when Egr-1 was deleted from experimental animals (Yan et al. 2000). An additional consequence of loss of barrier function is exposure of the previously sequestered and highly prothrombotic subendothelial matrix,



Endothelial Cells and Inflammation, Fig. 2 Endothelial cells maintain vascular homeostasis. (**a**) Endothelial cell factors affecting coagulation, fibrinolysis, and platelet aggregation. Under basal conditions, the endothelium promotes an anticoagulant surface in diverse ways. Endothelial activation or injury tips the balance toward fibrin accumulation (*tPA* tissue plasminogen activator, *uPA* urokinase plasminogen activator, *NO* nitric oxide; *PAI-1* plasminogen activator inhibitor-1; *vWF* von Willebrand factor). (**b**) Endothelial cell influence on vasomotor tone. Endothelial cells are necessary for vasodilatory and vasoconstrictor responses (*CO* carbon monoxide, *EDHF* endothelium-derived hyperpolarizing factor, *NO* nitric oxide, *ET-1* endothelin-1, *PDGF-B* platelet-derived growth factor-B, *VEGF* vascular endothelial growth factor)

rich in tissue factor and collagen. Notably, changes in barrier function are often reversible if the initial insult is removed.

Dynamic Regulation of Coagulation, Fibrinolysis, and Platelet Aggregation

Ordinarily, the endothelium is a nonthrombotic surface which maintains blood fluidity and vascular homeostasis. This is especially remarkable when considered in light of the fact that blood exposure to nearly all other surfaces results in coagulation. The endothelium maintains its *anticoagulant surface* under basal conditions in diverse ways (Fig. 2a). The presence of heparan sulfate on the surface of the endothelial cell, which acts as a cofactor for antithrombin III, results in the inactivation of thrombin. *Thrombin*, a serine protease which is activated by factor Xa, converts fibrinogen into *fibrin* and promotes coagulation. The endothelial cell membrane surface also contains thrombomodulin. *Thrombomodulin* alters the action of thrombin, such that thrombin becomes a potent activator of protein C, which has anticoagulant properties (Ten and Pinsky 2002). Activated protein C inhibits clotting by inactivating factors Va and VIIIa. The endothelium can produce tissue and urokinase-type plasminogen activators (tPA and uPA) to promote fibrinolysis by converting plasminogen to *plasmin*, should a thrombus begin to form. Endothelial cells also produce *plasminogen activator inhibitor-1* (PAI-1). Since PAI-1 is the major regulator of tPA and uPA, a dynamic balance is struck by

the endothelium, with net fibrinolytic activity dependent upon the relative activity of these factors.

Endothelial cell release of nitric oxide (NO) inhibits platelet activation and aggregation. Endothelial cells also produce eicosanoids, lipidic biomediators including *prostaglandins* and *thromboxane*. Prostacyclin (prostaglandin I_2 or PGI_2) prevents platelet activation and is a potent vasodilator. PGI_2 and thromboxane (TXA_2) act as physiologic antagonists. TxA_2, also produced by the endothelium, activates platelet aggregation and acts as a vasoconstrictor. The balance of TXA_2 and PGI_2 effectively modulates platelet aggregation. CD39 present on the surface of endothelial cells degrades ATP and ADP, both platelet agonists, thereby attenuating platelet activation.

Activation of the endothelium tips the homeostatic balance of the cellular phenotype toward a procoagulant state (Figs. 1 and 2a). The redundancy of multiple pathways leading to the same effect highlights the importance of the role of the endothelium in orchestrating a response to cellular damage. Tipping the balance in favor of procoagulability occurs in several ways. For example, activation of the endothelium by exposure to hypoxia results in the reduction of thrombomodulin levels at the endothelial surface. As a result, a procoagulant environment is created. *Tissue factor* is a transmembrane protein which under circumstances of vascular homeostasis is not expressed on endothelial cells. Instead, tissue factor is expressed on cells in the subendothelial layers of the vasculature. Injury to the blood vessel results in endothelial denudation and exposure of the blood to tissue factor. This is a potent stimulus of the extrinsic coagulation pathway. IL-1 and TNF stimulate endothelial cells to secrete tissue factor. Exposure of subendothelial tissue factor after endothelial injury is an additional factor which promotes a local coagulable environment. Mononuclear phagocytes recruited to the vessel wall during hypoxia are a source of procoagulant tissue factor (Karimova and Pinsky 2001). Further, hypoxia induces the transcription factor Egr-1, leading to increased tissue factor gene expression in the vessel wall.

The importance of the interaction between mononuclear cells and the endothelium is also apparent when examining the suppression of the fibrinolytic axis which occurs after endothelial cell activation. *Plasminogen activator inhibitor-1* (PAI-1) is responsible for the suppression of the conversion of plasminogen to plasmin. As a consequence, increased PAI-1 expression promotes thrombus accumulation. Hypoxia has been shown to increase PAI-1 transcription and mRNA stability (Karimova and Pinsky 2001). Transcription factors early growth response gene-1 (Egr-1), hypoxia-inducible factor-1α (HIF-1α), and CCAAT/enhancer binding protein alpha (C/EBP alpha) are quickly activated in hypoxia and are responsible for transcription and expression of PAI-1 (Liao et al. 2007). Therefore, via PAI-1, hypoxia promotes thrombus accumulation by reducing its dissolution. Mononuclear cells are the predominant source of PAI-1 expression under hypoxic conditions. Hypoxia also inhibits plasminogen activator gene (tPA and uPA) expression, promoting fibrin accumulation (Pinsky et al. 1998).

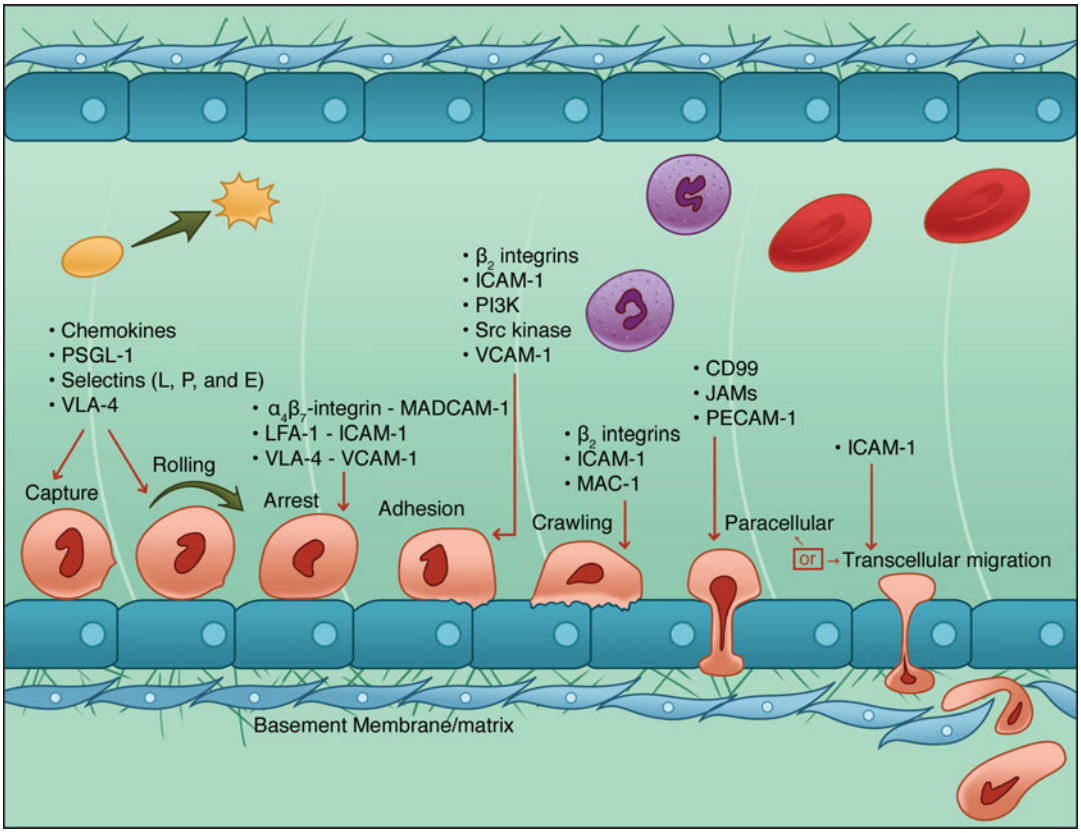
Activation of endothelial cells also drives the exocytosis of Weibel-Palade bodies (Pinsky et al. 1996). Endothelial cells contain *Weibel-Palade bodies*, intracellular organelles which store proteins including P-selectin, a leukocyte adhesion molecule, and procoagulant von Willebrand factor (vWF). Surface-immobilized vWF promotes platelet accumulation and thrombosis, by binding platelet glycoprotein receptors GP1bα and GPIIb/IIIa. Platelets home to sights of vascular injury, promoting thrombin generation and amplifying further platelet recruitment. Early in the course of systemic sclerosis, elevated vWF levels have been noted, representative of the endothelial activation and injury which occurs in this disease. In another illustration of the role of vWF, reperfusion injury is associated with increased platelet accumulation and thrombosis, in part as a consequence of precipitous decline in nitric oxide levels (Pinsky et al. 1994). This is in part due to the inhibitory effect of NO on the release of vWF from Weibel-Palade bodies under normoxic conditions (Matsushita et al. 2003).

Proinflammatory Cytokines

Cytokines are protein molecules secreted from numerous cell types, including macrophages and endothelial cells, and function to communicate signals from one cell to the next. Cytokines cause the quiescent endothelium to become activated and affect barrier function. One major group of cytokines is composed of a class of molecules, *interleukins*. Interleukin-1 (IL-1) was the first interleukin to be described, and other cytokines have since been identified that belong to the IL-1 family (Sims and Smith 2010). IL-1 is a particularly relevant target in autoimmune and chronic inflammatory diseases. It has been shown that T helper 17 cells require IL-1 signaling in order to differentiate from naïve T cells. The larger IL-1 family is composed of 11 molecules. These are IL-1α, IL-1β, IL-1Ra, IL-18, IL-33, and IL-1 F5-IL1F10. Expression levels and modes of activity differ between these highly similar interleukins. For example, IL-1β is produced by monocytes and macrophages and is able to circulate freely, whereas IL-1α is produced by a number of cells, including macrophages and endothelial cells, and is bound to the plasma membrane and tends to act locally. The two are also functionally different. For example, IL-1α is able to prime T cells during contact hypersensitivity, whereas IL-1β is involved in cell proliferation, differentiation, and apoptosis. IL-1Ra, on the other hand, can act to block the actions of IL-1α and IL-1β by binding their receptor, IL-1R1. This is an intriguing example of two countervailing forces, IL-1 and IL-1Ra, the net balance of which can either promote or inhibit inflammation. Importantly, IL-1 also influences coagulation. IL-1 induces coagulation and fibrin deposition. IL-1 induces the procoagulant cofactor tissue factor, while concomitantly blocking the protein C anticoagulant pathway and reducing thrombin-mediated protein C activation (Nawroth et al. 1986). IL-1 has also been shown (along with another cytokine, interferon-gamma or IFN-γ) to increase vascular permeability (Martin et al. 1988), thus demonstrating the integrative and critical role of cytokines for both immunologic activation and vascular endothelial function.

A subgroup of cytokines, chemotactic cytokines or chemokines, can influence the movement of leukocytes during inflammation (Fig. 3). After the innate immune response is provoked, chemokines signal the next step in progression of the response, promoting infiltration of inflammatory cells. Chemokines are classified into four families based on structural and functional differences. Of these four families, the largest is composed of the 27 identified *CC chemokines*, named for their common sequence of two adjacent cysteine residues near the amino terminus. Monocyte chemoattractant protein-1 (MCP-1) can recruit monocytes, dendritic cells, memory T cells, and basophils (Charo and Ransohoff 2006). This family of CC chemokines also includes macrophage inflammatory protein-1 (MIP-1) and RANTES (regulated upon activation, normal T cell expressed and secreted, otherwise known as CCL-5). *CXC chemokines* comprise another family of chemokines, named for an amino acid residue between the CC residue found in CC chemokines. This family of chemokines includes interleukin-8, or CXCL8, which is released by macrophages and endothelial cells and which can bind the receptors CXCR1 and CXCR2 to recruit polymorphonuclear cells as part of the innate immune response. A subfamily within the CXC chemokines is the *ELR + CXC chemokines*. These contain an ELR residue sequence (glutamate-leucine-arginine) near the N terminus and participate in wound healing and angiogenesis. Monocyte chemoattraction by chemokines involves a group of related chemokines, CCL2, 7, 8, and 13, as well as the ELR + CXC chemokines.

Other cytokines, such as interleukin-2 (IL-2), interferons, and tumor necrosis factor (TNF), have been studied as promoters of immune responses and found to play a role in autoimmune disease progression (O'Shea et al. 2002). Naïve T cells, after stimulation by an antigen, produce IL-2, which promotes clonal expansion as well as the production of other cytokines, including TNF. Within this clonal expansion, naïve T cells differentiate into one of two types. One group develops into TH1 cells under



Endothelial Cells and Inflammation, Fig. 3 Leukocyte adhesion. This cross section of a blood vessel shows the migration of a leukocyte from the capture phase along the endothelium to its complete migration past the basement membrane and smooth muscle layer. With cellular damage, activation of the endothelium, and the subsequent release of cytokines/chemokines such as IL-1β and TNF, leukocytes become activated. The activated endothelium possesses procoagulant properties, as shown by the activation of platelets. The increased expression of adhesion molecules (e.g., increased integrin-mediated binding) leads to arrest and firm adhesion of activated leukocytes. This transit across the endothelial barrier requires the involvement of molecules such as junctional adhesion molecules (e.g., JAM-A, B, C, ESAM). *PSGL-1* P-selectin glycoprotein ligand 1, *VLA-4* very late antigen 4, *LFA-1* lymphocyte function-associated antigen 1, *ICAM-1* intercellular adhesion molecule 1, *VCAM-1* vascular cell adhesion molecule 1, *MADCAM-1* mucosal vascular addressin cell adhesion molecule 1, *PI3K* phosphoinositide 3 kinase, *MAC-1* macrophage-1 antigen, *PECAM-1* platelet endothelial cell adhesion molecule, *CD99* cluster of differentiation 99 (MIC2), *JAM* junctional adhesion molecule

the control of IL-12, and the other group, TH2, under the control of IL-4. The TH1 subtype is most associated in the development of autoimmunity. For example, TH1 cells release interferon-γ (IFN-γ), resulting in elevated tissue levels of IL-4, which also modulates the TH1/TH2 balance and acts as an immune regulator, as do the cytokines IL-10 and transforming growth factor β (TGF-β). Blockade of these cytokines has resulted in increases in inflammation and autoimmunity. Cytokines also have broad effects on the endothelium. For example, TNF has been shown to increase vascular permeability in conjunction with VEGF (vascular endothelial growth factor) (Clauss et al. 2001). TNF acts in conjunction with IFN-γ to deactivate $\alpha_V\beta_3$ integrin, which is necessary for survival in angiogenic endothelial cells (Lejeune 2002).

Treatments have been developed which utilize cytokines as modulators of inflammatory diseases. For example, TNF-α, a cytokine secreted primarily from activated macrophages,

is a potent proinflammatory signal. It can stimulate the production of ELR + CXC chemokines and induces leukocyte adhesion to vascular endothelial cells. Neutralizing antibodies against TNF-α, or TNF inhibitors, are used to treat inflammatory diseases (Charo and Ransohoff 2006). Etanercept is a TNF inhibitor that is used to treat rheumatoid arthritis and is made as a fusion protein that links TNF receptor 2 to human IgG1. The soluble TNF2 receptor binds TNF competitively, and the IgG fusion element increases the serum half-life of the drug relative to other soluble receptors. Adalimumab, a human monoclonal antibody against TNF-α, is used to treat rheumatoid arthritis and Crohn's disease. Infliximab is a mouse-human chimeric antibody against TNF-α. Newer reports have revealed that the traditional view of proinflammatory cytokines (IFN-γ, IL-2, TNF) as inciters of autoimmunity and regulatory cytokines (IL-4 as protectors against autoimmunity) is too simplistic. Proinflammatory cytokines may demonstrate immunosuppressive roles; thus the idea has been introduced that the particular cytokine makeup, in concert, determines the progression of the disease state.

Cell Adhesion

The process of inflammatory cell adhesion to the vascular endothelium and eventual transendothelial migration (also termed extravasation) requires a tightly regulated series of changes on the part of both inflammatory cells and the endothelium. These changes include morphologic cytoskeletal remodeling to optimize adhesion (from rolling adhesion to firm adhesion), molecular signaling pathway activation, and redistribution and increased expression of adhesion molecules and their receptors to facilitate movement across the endothelium (Fig. 3). Movement across the endothelium can occur either between endothelial cells in a process called paracellular migration or through cells via the transcellular route. The specific process of extravasation varies between inflammatory disease states.

Activation of the endothelium leads to the upregulation of adhesion molecules that assist in inflammatory cell adhesion, such as E-selectin, ICAM-1, and VCAM-1. Endothelial cell secretion of chemokines CCL2, CCL5, CXCL10, and CX3CL1 attracts monocytes, dendritic cells (DCs), and T cells. Morphologic changes to the endothelial plasmalemma, such as the clustering of ICAM-1 and VCAM-1, as well as the directional expression of their respective receptors on the leading edge of inflammatory cells result in the generation of a molecular gradient that directs movement toward the endothelium (Nourshargh et al. 2010).

Integrins are highly involved in leukocyte recruitment, and the binding of integrins to their respective endothelial ligands is important for firm adhesion. Integrins comprise a large adhesion receptor family, and they are expressed as heterodimers with alpha and beta subunits, such as VLA-4 ($\alpha_4\beta_1$), LFA-1 ($\alpha_L\beta_2$), and Mac-1 ($\alpha_M\beta_2$). Leukocyte integrins are expressed in inactive states and undergo conformational changes upon leukocyte activation. Once adhesion is established on the vascular surface, lymphocytes begin to spread and crawl over endothelial cells through a series of morphologic changes involving cytoskeletal remodeling as well as clustering of integrins and adhesion molecules (Shulman et al. 2009). When engaged, integrins can also mediate signaling from the endothelial cell to the leukocyte in a process known as "outside-in signaling."

The upregulation of *junctional adhesion molecules* (JAMs) allows infiltrating inflammatory cells to pass across the tight junctions formed by the endothelial barrier. JAMs include a set of three closely related CTX proteins, JAM-A, JAM-B, and JAM-C, and also four other family members, ESAM, CAR, JAM-4, and JAM-L (Bradfield, ATVB 2007). The integrin LFA-1 ($\alpha_L\beta_2$) has been shown to bind JAM-A to mediate transmigration. Mice deficient in JAM-A have shown reduced leukocyte accumulation at sites of injury across a number of different injury models, including peritonitis, myocardial ischemia/reperfusion, hepatic ischemia/reperfusion,

and atherosclerotic vascular injury. The roles of JAM-B and JAM-C have also been explored; these JAMs relate with each other in multimeric interactions. JAM-B on endothelial cells binds the integrin VLA-4 and requires JAM-C expression on the infiltrating leukocyte.

Atherosclerosis is an example of a chronic inflammatory disease that illustrates various mechanisms of cellular adhesion and infiltration. In atherosclerosis, high circulating levels of low-density lipoproteins (LDL) carrying cholesterol trigger fatty plaque deposition. LDL particles accumulate subendothelially in the intima. Oxidative factors, such as reactive oxygen species, myeloperoxidases, and lipoxygenases, are released in the course of atherosclerotic progression. These factors lead to the formation of a varied group of modified LDL particles called oxidized LDL (oxLDL). The oxidation byproducts of these lipids are strong activating signals for endothelial cells and macrophages. As a result, there is an increase of inflammatory cell recruitment via an increase in adhesion molecules and cytokine release from the activated endothelium and macrophages. This type of chronic activation may be particularly important in diseases such as rheumatoid arthritis or systemic lupus erythematosus (SLE), which are associated with increased risk of atherosclerosis.

Inflammatory cells take up cholesterol through *pattern-recognition receptors* (PRR). Signaling through PRR results in upregulation of integrins (Hansson and Hermansson 2011) (Nourshargh et al. 2010). Intracellular PRRs also exist, and these are capable of aggregating into *inflammasomes*, aggregations of proteins that promote inflammatory processes such as the release of proinflammatory cytokines IL-1β and IL-18. Macrophages take up oxLDL via scavenger receptors (CD36, SRA-1, SRA-2, SR-B1, LOX-1, PSOX, MARCO), and their accrual of cholesterol then promotes the progression macrophages into foam cells that form in the core of the atherosclerotic lesion. *Toll-like receptors* (TLR) on macrophages also bind oxLDL and thus initiate signaling pathways that further promote atherosclerosis.

Leukocyte Activation

Leukocyte activation and transmigration is crucial for normal innate and adaptive immunity. During tissue injury or infection, a chemotactic gradient forms, recruiting inflammatory cells to the site of injury. An example of this is the production of monocyte chemotactic protein-1 (MCP-1), a chemoattractant protein. MCP-1 attracts monocytes, leukocytes, and dendritic cells to an injured tissue. Release of tumor necrosis factor-alpha (TNF-α) and IL-1, as well as complement activation, stimulates inflammatory cells.

Hypoxia is known to induce the endothelium to release IL-8, which plays an important role in polymorphonuclear leukocyte recruitment and activation (Oz et al. 1995).

Once at the site of injury, leukocytes first undergo rolling adhesion, mediated primarily by selectin expression on the surface of activated endothelial cells (Fig. 3). Endothelial selectins then engage their cognate ligands on the leukocyte surface, such as P-selectin glycoprotein ligand-1 (PSGL-1). This results in shape change, both of endothelial cells and leukocytes. These morphologic changes promote subsequent firm adhesion. In endothelial cells, engagement of adhesion receptors prompts the formation of docking structures around leukocytes. Docking structure formation at the apical surface of endothelial cells requires cytoskeletal scaffold rearrangement. These alterations in endothelial cell morphology complement the corresponding changes in leukocytes. As the leukocyte begins rolling along the endothelial cell surface, becoming activated, the cell transforms from a free-flowing round cell into a polarized cell with a leading edge, with adhesion molecules such as integrins clustered to facilitate transmigration. Activated endothelial cells secrete IL-1, which in turn increases expression of intercellular adhesion molecule-1 (ICAM-1), an adhesion molecule found on the surface of endothelial cells and leukocytes. This results in auto-amplification of adhesion receptor expression, leukocyte recruitment, and inflammation. Once activated, leukocytes adhere to vascular wall and

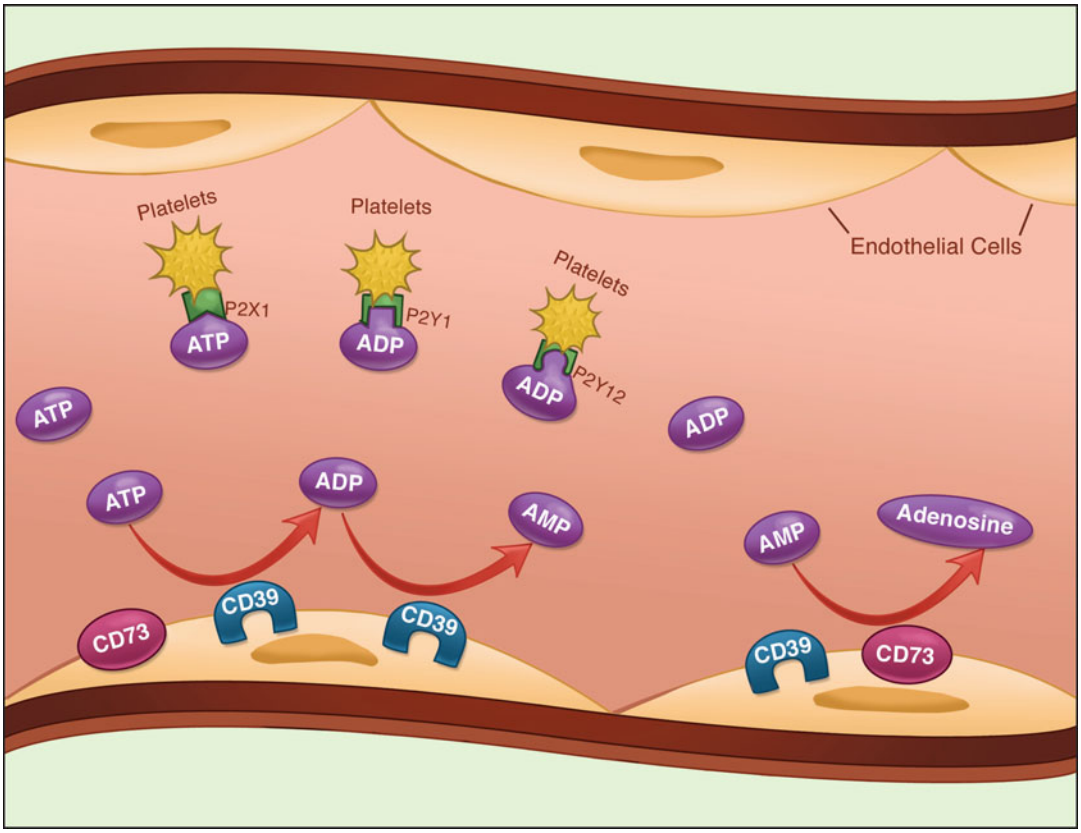
transmigrate. In the tissue, polymorphonuclear leukocytes and monocytes release cytotoxic enzymes, acids, and reactive oxygen species (Karimova and Pinsky 2001).

Reparative processes after infection, burns, or trauma depend on these events to promote removal or repair of damaged tissue. Thus, inflammation initiated by endothelial cells serves a physiologic purpose. It has been shown that distinct waves of inflammatory cell infiltration after an insult play important and differing roles in tissue repair. For example, in an animal model it was shown that after myocardial infarction, initial infiltration of a monocyte population which phenotypically exhibits phagocytic, proteolytic, and inflammatory functions occurs. This serves to remove necrotic tissue. This is followed by a monocyte population which characteristically displays attenuated inflammatory properties and promotes healing via myofibroblast accumulation and collagen deposition (Nahrendorf et al. 2007). This implies that the limitation of inflammatory cell infiltration after injury might stunt or slow wound healing.

Inflammation can become dysregulated due to positive feedback loops which result from cytokine release, adhesion molecule expression, cellular infiltration, and release of cytotoxic substances. If the insult is severe or prolonged, dysregulation of the inflammatory cascade may result in further damage to the parenchyma and endothelial cells themselves. Injured endothelial cells are themselves strong activators of the complement cascade, further leading to inflammation and injury. Some lupus patients have inherited deficiencies of C2, C4, or C1q. It has been hypothesized that this deficiency hampers the removal of circulating immune complexes and results in increased immune complex deposition, such as that which occurs in certain forms of lupus nephritis. Animal models have demonstrated that C3 deficiency protected from stroke, with a decreased brain infarct volume when compared with controls. C3-deficient mice manifested decreased granulocyte infiltration and reduced oxidative stress, highlighting the importance of C3 in brain inflammation (Mocco et al. 2006).

Dysregulated inflammation is common to many pathological conditions, from autoimmune disease to sepsis. In systemic lupus erythematosus (SLE), deposition of immune complex and complement activation are hallmarks of end-organ damage. Complement activation has been linked to the excess atherosclerosis seen in lupus, by stimulating endothelial cells and enhancing recruitment of leukocytes to the arterial wall (Narshi et al. 2011). Immune complexes stimulate endothelial cells to express VCAM-1, promoting monocyte infiltration (Narshi et al. 2011). In SLE-associated vasculitis, autoantibodies induce a proinflammatory and proadhesive endothelial cell phenotype through activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factor pathway (Narshi et al. 2011). Antiendothelial cell antibodies also induce upregulation of cell adhesion molecules such as E-selectin, ICAM-1, and VCAM-1 and enhance secretion of chemokines including MCP-1 and cytokines such as IL-1, IL-6, and IL-8 (Narshi et al. 2011). Antiendothelial antibodies are also thought to mediate endothelial cell apoptosis (Narshi et al. 2011).

Systemic sclerosis (SSc) is initiated by endothelial injury, and major features of this disease are the presence of mononuclear cell infiltrates and progressive structural defects in microvessels (Trojanowska 2010). SSc patients have been found to have a decrease in the expression of molecules involved in regulating endothelial cell-cell interactions, VE-cadherin and PECAM-1 (Trojanowska 2010). Decreased expression of these molecules may facilitate mononuclear cell infiltration in this disease. Friend leukemia virus integration 1 (Fli1) belongs to the Ets family of transcription factors and has an essential role in the regulation of genes encoding components of the extracellular matrix, including type I collagen. Importantly, Fli1 knockout mice show abnormal skin vasculature with many characteristics reminiscent of SSc, including a considerable decrease in the expression of molecules involved in endothelial cell-cell adhesion, VE-cadherin and PECAM1, as well as alterations in endothelial cell-pericyte



Endothelial Cells and Inflammation, Fig. 4 Ectonucleotidases limit inflammation CD39 phosphohydrolyzes extracellular ATP and ADP to AMP, and CD73 is responsible for the conversion of AMP to adenosine. ATP and ADP are released from activated platelets and from endothelial cells and other damaged cells following vascular injury. ATP binds the platelet receptor P2X1, and ADP binds the platelet receptors P2Y1and P2Y12 resulting in prothrombotic and proinflammatory effects. When adenosine binds its A3 and A2A receptors, anti-inflammatory and vasodilatory effects occur

interactions, including those involving the Tie 2 receptor (Trojanowska 2010).

In sepsis, systemic endothelial activation results in capillary leakage, hypotension, microvascular thrombosis, and tissue hypoxia. When the inflammatory response becomes dysregulated systemically, this can lead to multiorgan dysfunction and death. Augmentation of the inflammatory response in response to infection is necessary for host defense, and circulating levels of soluble adhesion molecules such as selectins, ICAM-1, and VCAM-1 are expected to increase in response to endothelial activation in infection. However, markedly elevated levels are present in those with sepsis and positively correlate with the severity of illness (Reinhart et al. 2002). The levels of these molecules have been found to be highest when severe sepsis was associated with a low probability of survival.

Of interest, self-regulation of leukocyte trafficking, moderated by leukocyte ectoenzymatic activity, may play an important role in limiting detrimental inflammation. Ectoenzymes are membrane proteins which have catalytically active sites in the extracellular environment. They are classified according to their enzymatic activities. Nucleotidases such as CD39 and CD73 are ectoenzymes involved in the extracellular metabolism of purine nucleotides including ATP (Fig. 4). They regulate inflammatory cell trafficking by modulating the levels of ATP, ADP, AMP, and adenosine at sites of

inflammation. ATP is released from dying cells at sites of injury. Binding of ATP and ADP to purinergic receptors results in prothrombotic and proinflammatory effects, while stimulation of certain adenosine receptors results in vasodilatory and anti-inflammatory effects.

In a murine model of cerebral ischemia, CD39 on monocytes and neutrophils was shown to regulate inflammatory cell sequestration into ischemic cerebral tissue, by catabolizing nucleotides released by injured cells (Hyman et al. 2009). It was shown that by this mechanism, inflammatory cells could self-regulate chemotaxis, adhesion, and transmigration. The importance of this is highlighted by the observation that cerebral infarct volumes were significantly larger in animals that did not express CD39. Cerebral infarct volumes were also shown to be larger in animals not expressing CD73, underscoring a critical role for CD73 as a modulator of brain inflammation (Petrovic-Djergovic et al. 2012). CD39 was shown to be the dominant Langerhans cell-associated ecto-NTPDase and had diverging roles in regulating inflammatory responses to environmental insults. CD39 deficiency resulted in attenuated T cell-mediated (allergic) contact hypersensitivity. In contrast, CD39-deficient animals exposed to irritant chemicals triggered rapid ATP and ADP release and demonstrated exacerbated skin inflammation in this model of irritant contact dermatitis (Mizumoto et al. 2002).

Vasomotor Tone

The endothelium plays a key role in regulating vascular tone and release of vasoactive agents. The endothelium is necessary for agents such as acetylcholine or bradykinin to cause vasorelaxation (Karimova and Pinsky 2001). Much like the endothelium maintains a nonthrombogenic surface which facilitates normal blood flow, under basal conditions, the vasoconstricting and vasodilating properties of the endothelium are in a dynamic balance (Fig. 2a). Because of the proximity of the endothelium to the underlying vascular smooth muscle, release of vasoactive signals from endothelium results in robust changes in vascular tone. Vasodilating substances released from the endothelium act in a paracrine manner. These include nitric oxide (NO), carbon monoxide (CO), prostacyclin, and endothelium-derived hyperpolarizing factor.

NO is a gaseous molecule with a short half-life in the blood stream, which serves as a key paracrine regulator of vasorelaxation. NO is synthesized from its precursor l -arginine by NO synthases. This reaction is dependent upon the presence of molecular oxygen. eNOS is the constitutive endothelial isoenzyme which produces NO. NO diffuses from endothelial cells and enters subjacent smooth muscle, where it binds guanylyl cyclase. This results in increased cGMP, which in turn mediates vasorelaxation through a reduction in intracellular calcium. NO also transcriptionally regulates the genes of vasoconstricting molecules, including endothelin-1 (ET-1) and platelet-derived growth factor-B (PDGF-B). As a result, NO both directly and indirectly regulates vasodilatation. During hypoxia, *eNOS* gene expression decreases, leading to decreased NO production by the endothelium (Karimova and Pinsky 2001). In turn, there is a decrease in guanylate cyclase activation and cGMP. In addition, during reoxygenation, NO is quenched by free radicals. This perpetuates the decrease in cGMP which occurred during the hypoxic period. NO-mediated vasodilation is impaired in many disease states, including atherosclerosis, hypertension, and diabetes.

CO is another endogenous gas that participates in vascular homeostasis. CO is produced during the breakdown of heme by the enzyme *heme oxygenase*. Hypoxia results in markedly increased expression of the inducible form of heme oxygenase, heme oxygenase-1. Similarly to NO, CO activates soluble guanylyl cyclase, albeit at a lower potency than does NO, resulting in increased cGMP. CO influences vascular tone and has noteworthy effects on hypoxia-induced transcription factors including Egr-1, as well as platelet aggregation and fibrinolysis (Karimova and Pinsky 2001; Mishra et al. 2006). The available data suggests that NO plays an important role in resting vascular tone, with CO playing

a less critical role under basal conditions. However, under conditions of hypoxia or ischemia/reperfusion, a decline in the bioavailability of NO and induction of heme oxygenase-1 may result in dominance of CO in influencing vasodilation.

Endothelium-dependent hyperpolarizing factor (EDHF) is induced by bradykinin and shear stress and, as its name implies, hyperpolarizes vascular smooth muscle. It is produced by the endothelium and dilates arteries by opening calcium-activated potassium channels. Prostacyclin is also endothelium derived and contributes to tonic coronary vasodilation. Prostacyclin is formed in vessel walls from the metabolism of arachidonic acid via cyclooxygenase.

The most potent vasoconstrictor synthesized by the endothelium is ET-1. Once released, ET-1 binds its receptor ET-A on vascular smooth muscle, increases intracellular calcium, and causes protein kinase C activation and myosin phosphorylation, leading to contraction (Karimova and Pinsky 2001). In contrast to the short-lived effects of vasodilators such as NO, constriction in response to ET-1 is prolonged. Hypoxia induces vasoconstriction and ET-1 production. It appears that under hypoxic conditions, the balance between vasodilating and vasoconstricting substances tips toward vasoconstriction (Figs. 1 and 2b). This occurs rapidly, within hours of acute hypoxia. Of note, NO and ET-1 production are mutually regulated, with NO suppressing ET-1 production. Other hypoxia-induced endothelium-released vasoconstricting substances include platelet-derived growth factor-B (PDGF-B) and vascular endothelial growth factor (VEGF). In diabetes, endothelial dysfunction contributes to increased hypertension and cardiovascular disease, in part due to increased endothelin, reduced prostacyclin activity, and abnormal NO biology (Orasanu and Plutzky 2009). Countering prostacyclin activity, thromboxane released by endothelial cells acts as a vasoconstrictor.

Growth Regulation

Endothelial proliferation is an important mechanism in angiogenesis which can be stimulated by endothelial activation. VEGF serves as a key growth factor in the regulation of ischemic angiogenesis. VEGFR-1 and VEGFR-2 are VEGF receptors expressed. On the endothelium and upregulated during hypoxia (Karimova and Pinsky 2001). Hypoxia-inducible factor-1 (HIF-1) is a transcription factor induced by hypoxia and influences the activation of the *VEGF* gene, among other genes including heme oxygenase-1 and iNOS (Karimova and Pinsky 2001). *VEGF* is also involved in vascular permeability, vasomotor tone, and regulation of fibrinolytic activity in hypoxia. Fibrin accumulates during hypoxia and serves as a matrix for subsequent angiogenesis, critical for restoration of blood flow to ischemic tissue (Karimova and Pinsky 2001). Fibroblast growth factor (FGF) and PDGF are also important growth factors. These growth factors have gained attention as therapeutic targets, both in restoring blood flow to ischemic organs and in limiting pathological angiogenesis in tumors. In systemic sclerosis, inflammatory signals resulting in endothelial activation lead to a rise in PDGF and TGF-β, triggering perivascular fibrosis.

Conclusion

The endothelium performs diverse functions and plays a critical role in maintaining vascular homeostasis. Endothelial cells perform the delicate task of balancing pro- and anticoagulant factors, inciting and tempering inflammation, and regulating cellular and molecular trafficking. In addition, the endothelium dynamically regulates vascular tone. Because of the numerous roles that the endothelium performs, endothelial activation is far reaching in its downstream effects. Endothelial activation impacts not only neighboring cells but the surrounding parenchyma as well. From an evolutionary perspective, these adaptable responses served to counter such varied insults as infection, trauma, and ischemia. On the other hand, endothelial cell activation and alterations in the basal vascular environment are a common denominator in many disease states, including those involved in atherosclerosis and

rheumatological diseases, among others. Looking toward the future, a challenge will be to develop clinical applications which fine-tune endothelial responses to injury. Clinically, targeting strategies which mimic the ideal endothelial response to injury is imperative. Development of approaches which would allow appropriate endothelial activation necessary for healing and host defense, while limiting detrimental, inappropriate activation, could have a vast impact on disease states from systemic sclerosis to atherosclerosis and stroke to sepsis.

Cross-References

- ▶ Atherosclerosis and Cytokines
- ▶ Autoantibodies to Endothelial Cells
- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Complement in Rheumatic Diseases
- ▶ Lymphocytes in Atherosclerosis
- ▶ Macrophages, Oxidative Stress, and Atherosclerosis
- ▶ Mechanisms of Endothelial Activation
- ▶ Neutrophils in Endothelial Damage
- ▶ NF-κB
- ▶ Nitric Oxide
- ▶ Platelets, Atherosclerosis, and Immunity
- ▶ Systemic Autoimmune Disease and Premature Atherosclerosis
- ▶ TGF-β
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Bradfield PF, Nourshargh S, Aurrand-Lions M, Imhof BA. Arterioscler Thromb Vasc Biol. 2007;27(10):2104–12.

Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med. 2006;354(6):610–21.

Clauss M, et al. A permissive role for tumor necrosis factor in vascular endothelial growth factor-induced vascular permeability. Blood. 2001;97(5):1321–9.

Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol. 2011;12(3):204–12.

Hyman MC, et al. Self-regulation of inflammatory cell trafficking in mice by the leukocyte surface apyrase CD39. J Clin Invest. 2009;119(5):1136–49.

Karimova A, Pinsky DJ. The endothelial response to oxygen deprivation: biology and clinical implications. Intensive Care Med. 2001;27(1):19–31.

Lejeune FJ. Clinical use of TNF revisited: improving penetration of anti-cancer agents by increasing vascular permeability. J Clin Invest. 2002;110(4):433–5.

Liao H, et al. Molecular regulation of the *PAI-1* gene by hypoxia: contributions of Egr-1, HIF-1alpha, and C/EBPalpha. FASEB J. 2007;21(3):935–49.

Martin S, et al. IL-1 and IFN-gamma increase vascular permeability. Immunology. 1988;64(2):301–5.

Matsushita K, et al. Nitric oxide regulates exocytosis by *S*-nitrosylation of *N*-ethylmaleimide-sensitive factor. Cell. 2003;115(2):139–50.

Mishra S, et al. Carbon monoxide rescues ischemic lungs by interrupting MAPK-driven expression of early growth response 1 gene and its downstream target genes. Proc Natl Acad Sci USA. 2006;103(13):5191–6.

Mizumoto N, et al. CD39 is the dominant Langerhans cell-associated ecto-NTPDase: modulatory roles in inflammation and immune responsiveness. Nat Med. 2002;8(4):358–65.

Mocco J, et al. Complement component C3 mediates inflammatory injury following focal cerebral ischemia. Circ Res. 2006;99(2):209–17.

Nahrendorf M, et al. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med. 2007;204(12):3037–47.

Narshi CB, Giles IP, Rahman A. The endothelium: an interface between autoimmunity and atherosclerosis in systemic lupus erythematosus? Lupus. 2011;20(1):5–13.

Nawroth PP, et al. Interleukin 1 induces endothelial cell procoagulant while suppressing cell-surface anticoagulant activity. Proc Natl Acad Sci USA. 1986;83(10):3460–4.

Nourshargh S, Hordijk PL, Sixt M. Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. Nat Rev Mol Cell Biol. 2010;11(5):366–78.

Orasanu G, Plutzky J. The pathologic continuum of diabetic vascular disease. J Am Coll Cardiol. 2009;53(Suppl 5):S35–42.

O'Shea JJ, Ma A, Lipsky P. Cytokines and autoimmunity. Nat Rev Immunol. 2002;2(1):37–45.

Oz MC, et al. Ischemia-induced interleukin-8 release after human heart transplantation. A potential role for endothelial cells. Circulation. 1995;92(9):428–32.

Petrovic-Djergovic D, et al. Tissue-resident ecto-5′ nucleotidase (CD73) regulates leukocyte trafficking in the ischemic brain. J Immunol. 2012;188(5):2387–98.

Pinsky DJ, et al. Cardiac preservation is enhanced in a heterotopic rat transplant model by supplementing

the nitric oxide pathway. J Clin Invest. 1994;93(5):2291–7.
Pinsky DJ, et al. Hypoxia-induced exocytosis of endothelial cell Weibel-Palade bodies. A mechanism for rapid neutrophil recruitment after cardiac preservation. J Clin Invest. 1996;97(2):493–500.
Pinsky DJ, et al. Coordinated induction of plasminogen activator inhibitor-1 (PAI-1) and inhibition of plasminogen activator gene expression by hypoxia promotes pulmonary vascular fibrin deposition. J Clin Invest. 1998;102(5):919–28.
Reinhart K, et al. Markers of endothelial damage in organ dysfunction and sepsis. Crit Care Med. 2002;30(Suppl 5):S302–12.
Shulman Z, et al. Lymphocyte crawling and transendothelial migration require chemokine triggering of high-affinity LFA-1 integrin. Immunity. 2009;30(3):384–96.
Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol. 2010;10(2):89–102.
Ten VS, Pinsky DJ. Endothelial response to hypoxia: physiologic adaptation and pathologic dysfunction. Curr Opin Crit Care. 2002;8(3):242–50.
Trojanowska M. Cellular and molecular aspects of vascular dysfunction in systemic sclerosis. Nat Rev Rheumatol. 2010;6(8):453–60.
Yan SF, et al. Egr-1, a master switch coordinating upregulation of divergent gene families underlying ischemic stress. Nat Med. 2000;6(12):1355–61.

Environment and Autoimmunity

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Synonyms

Autoimmune diseases; Environmental triggers

Definition

Genetic, epigenetic, hormonal, and other factors, including environmental agents, contribute to the development of autoimmune disease. For the preparation of this entry, we conducted a review of recent studies regarding autoimmunity and environmental exposure using the terms: autoimmune diseases, environmental factors related to smoking, sex hormones, estrogens, infections, and autoimmune triggers.

Autoimmune diseases account for approximately 0.05–5 % of diseases in the general population (Cooper and Stroehla 2003). Incidence rates vary among the autoimmune diseases, with estimates ranging from less than one newly diagnosed case of systemic sclerosis per 100,000 person-years to more than 20 cases of adult-onset rheumatoid arthritis per 100,000 person-years. Prevalence rates range from less than 5 per 100,000 (e.g., chronic active hepatitis, uveitis) to more than 500 per 100,000 (Graves' disease, rheumatoid arthritis, thyroiditis). At least some of these diseases are thought to be related to environmental triggers. Due to the difficulty of determining specific environmental exposures, it is challenging to define a potential role for specific environmental exposures in triggering autoimmune disease. The American College of Rheumatology (ACR) proposed three ways to link a particular environmental exposure to the development of an autoimmune disease, (Miller 2011; Miller et al. 2012):

1. Through epidemiological methods, including "Hill's criteria," i.e., strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy. These criteria further describe the potential causal link between autoimmune disease and environmental exposure as confident, likely, or unlikely (Miller et al. 2012).
2. By recognition of a new disease – This requires at least four of eight elements including at least three of five primary elements. The three primary elements should include temporal association, lack of likely alternative explanations, and at least one of the following: improvement with removal of the exposure (dechallenge), recurrence with re-exposure (rechallenge), and biologic plausibility (Miller et al. 2012). The remaining (secondary)

elements include prior reports of similar cases (analogy), dose responsiveness, and prior reports of identical cases (specificity). It was further suggested that this will be completed in four stages:
- Proposing the association
- Testing the association
- Defining criteria for the condition
- Refining criteria for the condition

3. Exposure inducing autoimmune disease in individuals – This can be tested by challenge, dechallenge, and rechallenge. If a link is suggested by challenge alone, the causation is described as possible. When a rechallenge is not possible due to the severity of the reaction, causation is considered probable. When suggested by all three (challenge, dechallenge, and rechallenge), causation is considered definite (Miller et al. 2012).

Despite the fact that there are still missing pieces of the puzzle, it is clear that the same agent can induce very different autoimmune disorders, possibly due to different genetic backgrounds, while multiple agents can produce a very similar clinical picture. The reporting of individuals who developed an autoimmune disease after a specific exposure that subsided after removal and reappeared after further exposure has added an insight on the role of many agents. These reports, together with in vitro studies, could further define the cellular players and the possible mechanisms of the suspected environmental agents involved in the induction or perpetuation of the autoimmune response.

Smoking

Cigarette smoke may trigger an autoimmune response by cellular necrosis caused by free radicals, release of pro-inflammatory cytokines, and DNA damage inducing genetic mutations, glycoprotein content inducing activation of lymphocytes, and modulation of hormones (Smyk et al. 2012). Smoking is associated with rheumatoid arthritis (RA), autoimmune thyroiditis, inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), multiple sclerosis (MS), and primary biliary cirrhosis (PBC) (Kakalacheva and Lünemann 2011; Miller 2011; Smyk et al. 2012; Moroni et al. 2012).

In a study of 241 patients with PBC, an increased odds ratio for the presence of disease was seen among smokers. In another study of 1,032 patients with PBC, similar findings were demonstrated (Smyk et al. 2012). A further association of PBC and smoking was demonstrated by a study of 2,258 patients, finding higher incidence of smokers among PBC patients (Smyk et al. 2012).

The role of smoking in the pathogenesis of multiple sclerosis (MS) was suggested by a meta-analysis of 14 studies (Kakalacheva and Lünemann 2011). Genetically susceptible individuals who smoked had a risk of developing MS that was 2.8 times higher than nonsmokers. The mechanism is unknown.

Irritants in the lung such as cigarette smoke may cause citrullination of lung proteins, triggering an autoimmune response in genetically susceptible individuals. This theory is supported by the association between RA and smoking, the presence of citrullinated proteins in the lung and in the inflamed joints of smokers with newly diagnosed RA, and positive anti-cyclic citrullinated peptide antibodies (anti-CCP) (Klareskog et al. 2011). A review of several studies demonstrated that current or past smoking increases the risk of RA, higher levels of rheumatoid factor, and increased disease severity (Pollard 2012).

In a meta-analysis, smokers have a higher risk of developing SLE compared with former or nonsmokers (Zandman-Goddard et al. 2012). Current, chronic smokers and former smokers are at a higher risk for having elevated titers of dsDNA antibodies, skin involvement, serositis, and neuropsychiatric involvement (Zandman-Goddard et al. 2012).

Hormone Replacement Therapy (HRT) and Sex Hormones

Sex hormones affect the immune system by influencing lymphocyte maturation, activation, synthesis of antibodies and cytokines, and modulating the innate and adaptive immunity components. In addition, sex hormones alter antigen-presenting cell (APC) numbers and

function, and regulation of dendritic cell differentiation (Moroni et al. 2012; Chighizola and Meroni 2012). Estrogens cause the stabilization of auto-reactive B cells via T helper 2 lymphocytes, the release of interleukins (IL), and other cytokines (Moroni et al. 2012; Chighizola and Meroni 2012).

Estrogens can also be found in the environment naturally (phytoestrogens, mycoestrogens), or industrially (xenoestrogens), in metals, plastics, detergents, surfactants, and pesticides (Chighizola and Meroni 2012). The exposure to these materials may directly affect the estrogen receptors or disturb the hormonal balance of the exposed individual (Chighizola and Meroni 2012). Several studies concerning exposure to estrogen have demonstrated effects on the immune system (Chighizola and Meroni 2012). In murine models, neonatal exposure to estrogen may cause impairment in the immune system, especially T cells (Chighizola and Meroni 2012).

Lupus is more frequent in females in the reproductive years, and in murine SLE models, estrogen administration causes an increase in titers of anti-DNA antibodies and worsening of immune complex glomerulonephritis (Moroni et al. 2012). Additional evidence is found in animal experiments showing improvement after oophorectomy and exacerbation following estrogen administration (Chighizola and Meroni 2012; Strickland et al. 2012).

In a murine animal experiment, genetically induced, susceptible lupus mice were treated with estrogen, causing the development of SLE, compared to controls that were not susceptible and had no signs of SLE (Strickland et al. 2012; Peeva and Zouali 2005). Examination of the kidneys of affected mice revealed glomerulonephritis with IgG (Strickland et al. 2012). It should be stated that the influence of estrogen was seen only in the female animals (Strickland et al. 2012).

Men with Klinefelter's syndrome (a condition associated with higher than normal estrogen levels) have an increased incidence of SLE (Chighizola and Meroni 2012).

One example of estrogen therapy associated with the development of autoimmune disease in humans was described in a man who underwent sex reassignment and later developed lupus erythematosus tumidus (Zandman-Goddard et al. 2007).

The effect of estrogen therapy in patients with PBC remains controversial. In a large, multicenter study of 1,032 patients with PBC, an association between hormone replacement therapy (HRT) and PBC was noted. However, bone loss and treatment with HRT is common among patients with PBC (Smyk et al. 2012). In another study, conflicting results were found. Of 222 patients with PBC, no association with HRT was found (Smyk et al. 2012). Estrogen therapy, including oral contraceptives, may trigger the development of lupus or a flare of the disease. However, treatment of SLE patients with HRT is relatively safe even though it may trigger mild flares. Recent research suggests that low dose estrogen oral contraceptives can be an acceptably safe option for SLE patients without antiphospholipid antibodies or overt antiphospholipid syndrome.

Infections

An association between infection and autoimmune disease is well established. A few examples are: streptococcal infection and rheumatic heart disease, hepatitis C virus with vasculitis, cryoglobulinemia and autoimmune hepatitis, enteroviruses with type 1 diabetes, Epstein–Barr virus with SLE, *Helicobacter pylori* with gastric autoimmunity, *Saccharomyces cerevisiae* with Crohn's disease, measles with multiple sclerosis and cytomegalovirus or Parvovirus B19 with systemic sclerosis (Smyk et al. 2012).

Urinary Tract Infections (UTI)

It has been demonstrated in several studies that *E. coli* can cross-react with pyruvate dehydrogenase complex (PDC)-E2 epitopes and can activate B and T cells in patients with PBC (Smyk et al. 2012). In a study of 241 patients with PBC, a high odds ratio of PBC was noted in patients with past recurrent UTI. Another study of 1,032 patients with PBC demonstrated a high incidence of recurrent UTI (Smyk et al. 2012). A study of 2,258 patients also demonstrated the association between PBC and recurrent UTI (Smyk et al. 2012).

Hepatitis Virus

In a study of 241 patients with PBC, an increased incidence of prior infection with hepatitis A was noted (Smyk et al. 2012). Several studies have demonstrated that early hepatitis A infection has a protective effect against the development of asthma. This was noted in a study of 1,600 Italian patients demonstrating a decreased likelihood of developing asthma in seropositive hepatitis A patients. The same result was found in a study of 30,000 individuals in the USA (Graham 2012).

Epstein Bar Virus (EBV)

EBV has been implicated in several autoimmune diseases, including SLE, MS, autoimmune thyroiditis (AT), RA, IBD, insulin-dependent diabetes mellitus (IDDM), Sjogren's syndrome (SjS), systemic sclerosis (SyS), myasthenia gravis, and autoimmune liver diseases (AiLD) (Rigopoulou et al. 2012). Several mechanisms are suspected to be involved in autoimmune disease associated with EBV. Among these is molecular mimicry cross-reacting with CYP2D6 and dysregulation of T-regulatory cells in genetically predisposed individuals (Rigopoulou et al. 2012).

Adolescents with EBV-associated infectious mononucleosis have a relative risk of 2.17 for developing MS according to a meta-analysis of 18 studies examining the relationship between MS and EBV (Kakalacheva and Lünemann 2011). Comparing MS patients and healthy controls, there was a significant difference in the risk of disease among those with past exposure to EBV especially in pediatric cases of MS (Kakalacheva and Lünemann 2011). A study of military personal demonstrated high EBV antibody (EBNA) titers in 69 patients with up to three times the risk for MS in such patients (Kakalacheva and Lünemann 2011). In several studies, specific EBNA-1 antibodies were found in MS patients, suggesting that an alteration in the immune system may lead to a higher risk of MS. There is limited evidence suggesting that molecular mimicry is responsible for the initiation of MS (Kakalacheva and Lünemann 2011).

AiLD includes several diseases with involvement of different cell types within the liver, including hepatocytes in autoimmune hepatitis (AIH), and cholangiocytes in PBC and primary sclerosing cholangitis (PSC) (Rigopoulou et al. 2012). In vitro studies have shown that EBV has the ability to induce B cell transformation. This may produce monoclonal autoantibodies and B cell proliferation and persistence as memory cells, even without additional stimulation (Rigopoulou et al. 2012).

The pathogenesis of AIH is unknown; however, there is some evidence of T-lymphocyte activation. AIH can be divided according to the autoantibody profile. Patients with type I AIH have antinuclear antibodies (ANA) and smooth muscle antibodies (SMA) while patients with type II disease have antibodies to liver kidney microsomal antigen type 1 (anti-LKM1) and anti-liver cytosol type 1 antibodies (anti-LC1) (Rigopoulou et al. 2012). There have been several case reports finding EBV antibodies, and EBV DNA in patients with AIH. (Rigopoulou et al. 2012). In PBC, autoantibodies target the epithelial bile ducts. Anti-mitochondrial antibodies (AMA) are pathognomonic. Patients with PBC have been found to have EBV DNA in peripheral blood cells in significantly higher amounts compared with control patients and those with other liver diseases (Rigopoulou et al. 2012). However, EBV DNA can be found in several autoimmune diseases and its presence is not specific to PBC (Rigopoulou et al. 2012).

In a study of 260 patients (120 of whom had lupus), lupus patients with evidence of prior EBV infection were more likely than those without EBV infection to have a mild form of SLE with skin and joint involvement and elevated anti-Ro antibodies. (Zandman-Goddard et al. 2009 -Ann NY Sci Acad).

Parvovirus B19

Parvovirus B19 infection can mimic autoimmune disease as it may cause rash, arthritis, and thrombocytopenia. Furthermore, it has been implicated as a trigger in patients who develop RA, SLE, and vasculitis. Direct effects on the immune system include molecular mimicry (Lunardi 2008; Miller 2011).

Human Herpes Virus 6 (HHV6)

HHV6 infects oligodendrocytes and microglia cells and its complications may include central

nervous system (CNS) involvement (Kakalacheva and Lünemann 2011). In several studies, the virus has been isolated from CNS tissue of MS patients and from MS plaques (Kakalacheva and Lünemann 2011). Further evidence of HHV6 involvement in MS is that the myelin basic protein (MBP) sequence is similar to sequences in HHV6, raising the possibility of developing MS through molecular mimicry. This is suggested by the elevated T-cell response targeting MBP and the U24 proteins of the HHV6 (Kakalacheva and Lünemann 2011).

Helminths

Parasitic infection may be protective against the development of autoimmune diseases (Zandman-Goddard and Shoenfeld 2009- Lupus). For example, there is an inverse correlation between helminthic infection and the development of SLE (Zandman-Goddard and Shoenfeld 2009-Lupus).

In studies of mouse models of MS, exposure to helminths was associated with improvement in the disease (Kakalacheva and Lünemann 2011). This was further demonstrated in a study of 12 MS patients infected with helminths who noted a decrease in disease activity and in radiographic findings; the mechanism appeared to be downregulation of pro-inflammatory cytokines. These improvements reversed after the parasitic infection was treated (Correale and Farez 2011; Zandman-Goddard and Shoenfeld 2009-Lupus). Further evidence of potentially protective effect of helminthic infection for the development of MS includes the observation that geographic regions where parasitic infestation with *T. trichiura* is common have a lower incidence of MS compared with areas where the prevalence of this parasite is low (Correale and Farez 2011).

A number of studies report that children dewormed in Vietnam and Venezuela had an increased incidence of asthma. A reverse correlation was also observed between the severity of symptoms among children with asthma and the presence of helminthic infection (Correale and Farez 2011).

The hygiene hypothesis may account, at least in part, for these observations. This hypothesis states that the decrease in exposure to microbial environmental elements plays a key role in the increase in autoimmune diseases found in developed countries (Graham 2012). For example, the theory states that due to dietary changes, altered interactions between the immune system and gut flora trigger autoimmune reactions which could include disorders such as diabetes (Graham 2012). This may be mediated by toll-like receptors as knockout mice lacking certain toll-like receptors do not develop diabetes (Graham 2012). An early exposure to hepatitis A virus is associated with protection against allergic disorders via T-cell modulation (Graham 2012). Early exposure to coxsackievirus B and rotavirus may have a protective effect for autoimmune disease development; however, due to the late exposure in developed countries, a matured immune system may react with an autoimmune reaction instead.

Increases in saponins, lectins, gliadin, and capsaicin ingestion lead to increased intestinal permeability and increased exposure to endotoxins and cross-reactive epitopes (Graham 2012). This observation is supported by studies of germ-free mouse models and the effects of bacterial exposure. Exposing these mice to intestinal flora, *Clostridium* species, and *Bacteroides fragilis* brought the induction of T-regulatory cells in the gut. Segmented filamentous bacterium, in particular, causes T helper 17 to induce an autoimmune reaction (Graham 2012). Another study suggesting the importance of exposure to infectious agents found that the microbiota of children in developing countries were highly diverse compared with children in a developed country (Graham 2012). Of note, individuals with Crohn's disease tend to have low microbiota diversity (Graham 2012).

Several studies have linked the early exposure to pathogens with the risk for developing MS. This strengthens the notion that early exposure to pathogens is required for the proper regulation of the immune system. A further decreased risk was shown in children attending day care or a household with other siblings (Conradi et al. 2011).

Influenza H1N1 Virus

Narcolepsy is caused by the loss of hypocretin/orexin neurons in the hypothalamus; this may be

the result of an autoimmune process. There is no direct evidence of autoantibodies against hypocretin cell antigens; however, antibodies against Tribbles homolog 2 (TRIB2) protein produced by the hypocretin cells have been demonstrated, which may explain the hypocretin cell damage (Han et al. 2011). In a study examining narcolepsy in children, there was a ninefold increased risk of narcolepsy following H1N1 influenza vaccination with squalene/*a*-tocopherol adjuvant (Han et al. 2011). This finding was supported by an additional study finding a 6.6-fold increased risk for narcolepsy following H1N1 vaccination (Correale and Farez 2011). In a study of 629 patients with narcolepsy, there was a sevenfold increase in narcolepsy during the year of the H1N1 outbreak and a fourfold increase among those patients who received vaccination (Han et al. 2011). There was a clear correlation between upper respiratory infection and narcolepsy onset; moreover, the diagnosis of narcolepsy was associated with infection with *Streptococcus pyogenes* and anti-streptolysin O (ASO) antibodies (Han et al. 2011). Despite these findings, a recent study showed a similar incidence of narcolepsy prior to and after the H1N1 pandemic years (Han et al. 2011).

Helicobacter pylori (H. pylori)

Individuals diagnosed with *H. pylori* infection may have an increased risk of autoimmune disease (Hasni 2012). This could be due to an effect of bacteria on the innate immune system, causing an anti-inflammatory response via the toll-like receptors and via the inhibition T helper cell proliferation (Hasni 2012). Despite this, there is evidence of autoimmunity triggered by *H. pylori* such as molecular mimicry causing the formation of autoantibodies, including IgM-rheumatoid factor, anti-single-stranded DNA antibody, and anti-phosphatidylcholine antibody (Hasni 2012).

There is also evidence associating *H. pylori* with (idiopathic thrombocytopenic purpura (ITP). Improvement in ITP patients was noticed after eradication of the bacteria, although the mechanism is unknown (Hasni 2012).

Silica and Silicone Breast Implant Surgery

Silica has been shown to interact with immune cells. Silica acts as a pro-inflammatory substance inducing the complement cascade activation, triggering the production of autoantibodies, inducing the formation of immune complexes, and deregulating the T helper/regulatory cell ratio (Smyk et al. 2012). Occupational exposure to silica has been linked to SLE, RA, vasculitis, and SyS (Miller 2011; Smyk et al. 2012).

A meta-analysis of studies found that the risk of developing SLE and RA was increased among silica-exposed individuals compared with non-exposed individuals. (Pollard 2012).

Although controversial, a number of reports have suggested a link between the development of myositis and SyS and silicone implants (Miller 2011). Slow release of silicone from the implants by migration of silicone through the capsule and into systemic circulation may increase the risk of developing an autoimmune disease. Silicone antibodies have been detected in patients with silicone implants (Lidar et al. 2012). Mice with silicone implants developed anti-ds DNA antibodies and rheumatoid factor. The same findings can be seen in women with silicone breast implants (Lidar et al. 2012). However, large epidemiological studies have found no significant association between silicone implants and the development of autoimmune disease (Lidar et al. 2012).

Solvents

Exposure to solvents is associated with SyS (Smyk et al. 2012; Pollard 2012). A meta-analysis of 11 studies examining the association between trichloroethylene solvent and SyS noted an odds ratio of 2.5 in males (Pollard 2012). Solvents may also be an occupational or recreational environmental trigger of SLE (Zandman-Goddard et al. 2012).

Vitamin D

Vitamin D deficiency is global and has a direct effect on the immune system. Autoimmune diseases associated with vitamin D deficiency include IDDM, MS, IBD, SLE, RA, and celiac disease (Smyk et al. 2012).

Epidemiologic studies have reported a lower risk of MS in countries with higher sun exposure (Kakalacheva and Lünemann 2011). Further involvement of vitamin D in MS is suggested by the finding of higher vitamin D consumption and higher exposure to sunlight in regions where MS is less common. (Kakalacheva and Lünemann 2011). In an international study of 40,000 patients with MS, prenatal sunlight exposure was associated with a decreased frequency of MS (Kakalacheva and Lünemann 2011). A study that correlated vitamin D levels and self-reported sun exposure demonstrated a decreased risk of MS in those reporting the highest sun exposure. (Kakalacheva and Lünemann 2011). Vitamin D deficiency has also been associated with relapses of MS, although in one study, no significant difference in vitamin D or its metabolites was found in patients with MS compared to the control group (Kakalacheva and Lünemann 2011). Vitamin D may have a regulatory effect on pro-inflammatory cytokines inhibiting T-cell activation and directly affecting T cells via the vitamin D receptor (Kakalacheva and Lünemann 2011).

Vitamin D has an anti-inflammatory effect through its ability to elevate the expression of T-regulatory cells and inhibit the maturation of dendritic cells (Milliken et al. 2012).

In experimental encephalitis models, in vivo vitamin D supplementation was associated with decreased auto-antigen production and an increase in T-reg cells (Milliken et al. 2012). Furthermore, in animal studies, administration of high dose vitamin D was associated with immunosuppressive effects (Milliken et al. 2012).

In the vitamin D receptor/IL-10 double knockout mouse, a severe form of IBD was noted. Furthermore, vitamin D deficiency alone leads to a more severe form of IBD in experimental models (Cantorna 2012).

Decreased levels of vitamin D are common among patients with SLE and correlate with disease severity (Hamza et al. 2011; Amital et al. 2010).

Cosmetics

Although controversial, xenobiotics (found in hair dyes and nail polish) have been linked with the development of PBC. This may be due to immune activation against self-antigens, perhaps via alteration of mitochondrial antigens (Smyk et al. 2012; Pollard 2012; Cooper et al. 2010). Anecdotes of exposure to cosmetics (whitening skin creams, lipstick, hair dyes) and the development of SLE have been described. Exposure to tanning beds may cause an exacerbation of SLE (Zandman-Goddard et al. 2012).

Metals and Toxins

Some have suggested that aluminum is a triggering factor for Crohn's disease through an interplay between aluminum and colonic bacteria (Lerner 2012). Exposure to aluminum can be from a variety of sources:

- In the soil due to changes in soil acidity (Lerner 2012),
- Occupational exposure in melting and mining industries (Lerner 2012),
- Food – Additives, purified drinking water, processed cheese, non-dairy creamers, powdered milks or food, baking powder, cake mixes, pancakes, sugars, soy-based milk products, chewing gum, rice and noodles, vending machine drinks, and icing sugar (Lerner 2012),
- Iatrogenic exposure – dialysates, phosphate, binders, antacids, intravenous solutions, urinary bladder irrigation fluids, bone and dental cements, buffered aspirins, antidiarrheal and antihemorroidal medications, vaccine adjuvants, and toothpaste (Lerner 2012).

The absorption of the aluminum in the digestive tract has been demonstrated in several studies which demonstrates aluminum presence in the pigmentation of colonic Peyer's patches and in submucosal macrophages (Lerner 2012). In animal studies, dietary aluminum is associated with bowel inflammation.

In a case report, an RA patient improved after receiving chelating therapy for suspected metal intoxication (Bamonti et al. 2011). Among patients with MS, an increased excretion of aluminum was found in a few cases (Exley et al. 2006).

A study examining a potential link between mercury exposure and autoimmune disease demonstrated a clear association between mercury

exposure from mining, and skin creams and the formation of antinuclear autoantibodies (Pollard 2012).

In mouse models of SLE, pesticides were associated with the presence of ANA. In humans, ANA positivity may be linked with prolonged exposure to pesticides (Pollard 2012). Pesticides were also associated with increased risk for RA in the analysis of the Women's Health Initiative Observational Study (Parks et al. 2011).

Although the increased incidence of IDDM is worldwide, individuals who migrate to countries with a high incidence of IDDM have an increased risk of developing the disease. This supports the suggestion that environmental factors may trigger IDDM (Howard and Lee 2012). Studies have demonstrated correlations between the incidence of IDDM and the presence of nitrate compounds, persistent organic pollutants, ozone, sulfate, and polychlorinated biphenyls. Polychlorinated biphenyls were also found in high levels among pregnant women with IDDM. Elevated titers of glutamic acid decarboxylase (GAD) antibodies were found in workers exposed to polychlorinated biphenyls (Howard and Lee 2012). Bisphenol A, phthalates, and some pesticides may also be responsible for cases of autoimmune disease; of note, the autoimmune effect may occur at doses considered below the hazard level, influencing the endocrine system in a "U" shaped dose–response curve (Howard and Lee 2012).

Oleic acid anilide (OAA) exposure can trigger an autoimmune response and autoantibody production in animal experiments. This followed descriptions of the toxic oil syndrome associated with exposure to rapeseed oil denatured with aniline in 1981 (Germolec et al. 2012).

In 1989, an exposure to L-Tryptophan caused eosinophilia-myalgia syndrome with cutaneous manifestations of SyS. Animal experiments attempting to replicate the syndrome had only partial success, suggesting additional factors play a role (Germolec et al. 2012).

Ultraviolet Light

A link between MS and UV radiation is suggested by the relationship between the prevalence of MS and the latitude gradient which demonstrates an increased risk of up to 20 times with larger distances from the equator (Pollard 2012; Simpson et al. 2011). In addition, individuals at high altitude receiving more UV radiation have a lower incidence of MS (Schwalfenberg 2012).

Narrowband UVB therapy has been shown to exert immunosuppressive effects (Milliken et al. 2012). This can be explained by the direct effects of UV light inducing T-reg cells and through effects on vitamin D (Milliken et al. 2012). This can also be demonstrated in the treatment of immune-mediated dermatoses. In a study exposing patients to UVB light, an increase in peripheral T-reg cells was noted (Milliken et al. 2012).

Exposure to different types of UV light has a variable impact on cutaneous and systemic lupus. While UVA2 and UVB can exacerbate the disease, UVA1 can be protective (de Jong et al. 2012). Not only may sunlight cause exacerbations in SLE, but fluorescent light can also cause DNA damage and trigger SLE activity. Ironically, avoidance of light causes a decrease in vitamin D which may affect disease activity as well (de Jong et al. 2012).

Drugs

There are many drugs that are suspected of causing or contributing to autoimmune disease; however, for most, there is no definitive evidence for a direct, causal role (Miller 2011).

Drug induced lupus erythematosis (DILE) is the most common autoimmune disease triggered by drug therapy. It can be induced by many different types of drugs. The exact mechanism for this is not completely understood; however, it has been shown that hydralazine causes inhibition of DNA methyltransferase, causing overexpression of T-cell immune genes, leading to lupus-like disease (Strickland et al. 2012). Therefore, in genetically predisposed individuals, factors influencing DNA methyltransferase may cause lupus-like illness. DILE is usually characterized by anti-histone autoantibodies (rather than double-stranded DNA antibodies) and a mild form of disease. More severe drug-induced lupus may be triggered by anti-TNF biologic therapy.

These patients may develop renal and central nervous system involvement and antibodies to double-stranded DNA similar to SLE rather than DILE (Zandman-Goddard et al. 2012).

Statins may have an immunosuppressive or protective effect on autoimmune disease, including RA. Patients consistently taking statins for hypercholesterolemia had a lower prevalence of RA compared to those not adhering to the treatment. A hazard ratio of 0.58 for the development of RA was found in a study of 2,11,627 patients on long-term statin treatment (Chodick et al. 2010).

Although the evidence is scant, statins may trigger a lupus-like disease (de Jong et al. 2011). Several case reports describe lupus-like disease following statin treatment; however, in a study examining the World Health Organization reports of lupus-like disease associated with statin use, only 4.3 % out of 3,362 had elevated titers of antinuclear antibodies (ANA) (de Jong et al. 2011).

Ionizing Radiation

Patients treated with radiation therapy have an increased risk of developing Graves' disease (Pollard 2012). In a study of environmental radiation exposure in regions exposed to atomic bombs or nuclear reactor catastrophes, an increased incidence of AT has been noted (Pollard 2012).

Autoimmune Syndrome Induced by Adjuvants (ASIA) Syndrome

The ASIA syndrome is a constellation of autoimmune diseases triggered by adjuvant exposure. Diagnostic criteria were suggested composed of major and minor criteria (Shoenfeld and Agmon-Levin 2011):

- Exposure to an external stimuli (infection, vaccine, silicone, adjuvant) prior to clinical manifestations,
- The appearance of typical clinical manifestations: myalgia, myositis, or muscle weakness, arthralgia and/or arthritis, chronic fatigue, nonrestorative sleep, or sleep disturbances, neurological manifestations (especially associated with demyelination), cognitive impairment, memory loss, pyrexia, dry mouth,
- Removal of inciting agent induces improvement,
- Typical biopsy of involved organs.

Minor criteria:

- The appearance of autoantibodies or antibodies directed at the suspected adjuvant,
- Other clinical manifestations (i.e., irritable bowel syndrome),
- Specific HLA (e.g., HLA DRB1, HLA DQB1)
- Evolution of the symptoms into an established autoimmune disease (e.g., MS, SjS)

To establish the diagnosis of ASIA, a patient should meet at least two major criteria or one major and two minor criteria.

ASIA syndrome includes siliconosis, macrophagic myofasciitis (MMF) syndrome, Gulf war syndrome (GWS), and post-vaccination phenomena.

Adjuvants augment the activities of dendritic cells (DCs), lymphocytes, macrophages and activate the intracellular Nalp3 inflammasome system. In animal studies, adjuvants induce lupus-like disease with lupus autoantibodies and arthritis (Shoenfeld and Agmon-Levin 2011).

Prior events of immunization and pathologic conditions are known, such as swine flu vaccine outbreak of Guillain-Barré syndrome (GBS), transverse myelitis following oral polio vaccine, arthritis following diphtheria-tetanus-pertussis (DTP) and autoimmune thrombocytopenia after measles-mumps-rubella (MMR) vaccine (Shoenfeld and Agmon-Levin 2011).

MMF syndrome was found to be the result of persistence of aluminum adjuvant at the site of inoculation months and even 8–10 years following immunization in genetically susceptible subjects (Shoenfeld and Agmon-Levin 2011).

GWS occurred following multiple vaccinations including anthrax vaccine in which aluminum hydroxide and squalene served as adjuvants. In a study of 144 Gulf war-era veterans, 95 % of overtly ill deployed GWS patients had antibodies to squalene and 100 % of GWS patients were immunized to anthrax. Of deployed veterans without signs and symptoms of GWS, none had

antibodies to squalene. Neither patients with idiopathic autoimmune disease nor healthy controls had detectable serum antibodies to squalene (Shoenfeld and Agmon-Levin 2011; Asa et al. 2000).

Siliconosis is thought to develop after exposure to silicone with symptoms of autoimmune disease without fulfilling diagnostic criteria of specific autoimmune disease (Lerner 2012; Shoenfeld and Agmon-Levin 2011).

Conclusion

- Autoimmune diseases account for approximately 0.05–5 % of diseases in the general population.
- Environmental factors play an important role in the pathogenesis in genetically susceptible individuals.
- Smoking, hormone replacement therapy, sex hormones, vitamin D deficiency, toxins, heavy metals, solvents, UV radiation, and drugs are all implicated as triggers of autoimmune disease.
- The ASIA syndrome is a new entity involving environmental agents that may trigger the development of autoimmune manifestations.

Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Animal Models of Autoimmune Hepatitis
- ▶ Autoimmune Hepatitis
- ▶ Autoimmune Hepatitis: Pathogenesis, Association with Other Syndromes
- ▶ Discoid Lupus
- ▶ Gestational Alloimmune Liver Disease
- ▶ Immune Responses to the Hepatitis C Virus
- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Skin in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Gender and Hormone Influences
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Amital H, Szekanecz Z, Szücs G, Dankó K, Nagy E, Csépány T, et al. Serum concentrations of 25-OH vitamin D in patients with systemic lupus erythematosus (SLE) are inversely related to disease activity: is it time to routinely supplement patients with SLE with vitamin D? Ann Rheum Dis. 2010;69:1155–7.

Asa PB, Cao Y, Garry RF. Antibodies to squalene in Gulf War syndrome. Exp Mol Pathol. 2000;68:55–64.

Bamonti F, Fulgenzi A, Novembrino C, Ferrero ME. Metal chelation therapy in rheumatoid arthritis: a case report. Biometals. 2011;24:1093–8.

Cantorna MT. Vitamin D, multiple sclerosis and inflammatory bowel disease. Arch Biochem Biophys. 2012;523:103–6.

Chighizola C, Meroni PL. The role of environmental estrogens and autoimmunity. Autoimmun Rev. 2012;11:A493–501.

Chodick G, Amital H, Shalem Y, Kokia E, Heymann AD, Porath A, et al. Persistence with statins and onset of rheumatoid arthritis: a population-based cohort study. PLoS Med. 2010;7:e1000336.

Conradi S, Malzahn U, Schröter F, Paul F, Quill S, Spruth E, et al. Environmental factors in early childhood are associated with multiple sclerosis: a case–control study. BMC Neurol. 2011;11:123.

Cooper GS, Stroehla BC. The epidemiology of autoimmune diseases. Autoimmun Rev. 2003;2:119–25.

Cooper GS, Wither J, Bernatsky S, Claudio JO, Clarke A, Rioux JD, et al. Occupational and environmental exposures and risk of systemic lupus erythematosus: silica, sunlight, solvents. Rheumatology. 2010;49:2172e80.

Correale J, Farez MF. The impact of parasite infections on the course of multiple sclerosis. J Neuroimmunol. 2011;233:6–11.

de Jong HJ, Tervaert JW, Saldi SR, Vandebriel RJ, Souverein PC, Meyboom RH, et al. Association between statin use and lupus-like syndrome using spontaneous reports. Semin Arthritis Rheum. 2011;41:373–81.

de Jong HJ, Klungel OH, van Dijk L, Vandebriel RJ, Leufkens HG, van der Laan JW, et al. Use of statins is associated with an increased risk of rheumatoid arthritis. Ann Rheum Dis. 2012;71:648–54.

Exley C, Mamutse G, Korchazhkina O, Pye E, Strekopytov S, Polwart A, et al. Elevated urinary excretion of aluminium and iron in multiple sclerosis. Mult Scler. 2006;12(5):533–40.

Germolec D, Kono DH, Pfau JC, Pollard KM. Animal models used to examine the role of the environment in the development of autoimmune disease: findings from an NIEHS Expert Panel Workshop. J Autoimmun. 2012;39:285–93.

Graham AWR. Hygiene hypothesis and autoimmune diseases. Clin Rev Allergy Immunol. 2012;42:5–15.

Hamza RT, Awwad KS, Ali MK, Hamed AI. Reduced serum concentrations of 25 hydroxy vitamin D in Egyptian patients with systemic lupus erythematosus:

relation to disease activity. Med Sci Monit. 2011;17: CR711–8.
Han F, Lin L, Warby SC, Faraco J, Li J, Dong SX, et al. Narcolepsy onset is seasonaland increased following the 2009 H1N1 pandemic in China. Ann Neurol. 2011;70:410–7.
Hasni SA. Role of *Helicobacter pylori* infection in autoimmune diseases. Curr Opin Rheumatol. 2012;24:429–34.
Howard SG, Lee DH. What is the role of human contamination by environmental chemicals in the development of type 1 diabetes? J Epidemiol Community Health. 2012;66:479–81.
Kakalacheva K, Lünemann JD. Environmental triggers of multiple sclerosis. FEBS Lett. 2011;585(23):3724–9.
Klareskog L, Malmstrom V, Lundberg K, Padyukov L, Alfredsson L. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. Semin Immunol. 2011;23:92–8.
Lerner A. Aluminum as an adjuvant in Crohn's disease induction. Lupus. 2012;21:231–8.
Lidar M, Agmon-Levin N, Langevitz P, Shoenfeld Y. Silicone and scleroderma revisited. Lupus. 2012;21:121–7.
Lunardi C, Tinazzi E, Bason C, Dolcino M, Corrocher R, Pucceti A. Human parvovirus b19 infection and autoimmunity. Autoimmun Rev. 2008;8:116–20.
Miller FW. Environmental agents and autoimmune diseases. Adv Exp Med Biol. 2011;711:61–81.
Miller FW, Pollard KM, Parks CG, Germolec DR, Leung PS, Selmi C, et al. Criteria for environmentally associated autoimmune diseases. J Autoimmun. 2012;39:253–8.
Milliken SV, Wassall H, Lewis BJ, Logie J, Barker RN, Macdonald H, et al. Effects of ultraviolet light on human serum 25-hydroxyvitamin D and systemic immune function. J Allergy Clin Immunol. 2012;129:1554–61.
Moroni L, Bianchi I, Lleo A. Geoepidemiology, gender and autoimmune disease. Autoimmun Rev. 2012;11: A386–92.
Parks CG, Walitt BT, Pettinger M, Chen JC, de Roos AJ, Hunt J. Insecticide use and risk of rheumatoid arthritis and systemic lupus erythematosus in the Women's Health Initiative Observational Study. Arthritis Care Res (Hoboken). 2011;63:184–94.
Peeva E, Zouali M. Spotlight on the role of hormonal factors in the emergence of autoreactive B-lymphocytes. Immunol Lett. 2005;101:123–43.
Pollard KM. Gender differences in autoimmunity associated with exposure to environmental factors. J Autoimmun. 2012;38:J177–86.
Rigopoulou EI, Smyk DS, Matthews CE, Billinis C, Burroughs AK, Lenzi M, et al. Epstein-Barr virus as a trigger of autoimmune liver diseases. Adv Virol. 2012;2012:987471.
Schwalfenberg GK. Solar radiation and vitamin D: mitigating environmental factors in autoimmune disease. J Environ Public Health. 2012;2012:619381.
Shoenfeld Y, Agmon-Levin N. 'ASIA' – autoimmune/inflammatory syndrome induced by adjuvants. J Autoimmun. 2011;36:4–8.
Simpson Jr S, Blizzard L, Otahal P, Van der Mei I, Taylor B. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. J Neurol Neurosurg Psychiatry. 2011;82:1132–41.
Smyk D, Rigopoulou EI, Baum H, Burroughs AK, Vergani D, Bogdanos DP. Autoimmunity and environment: am I at risk? Clin Rev Allergy Immunol. 2012;42:199–212.
Strickland FM, Hewagama A, Lu Q, Wu A, Hinderer R, Webb R, et al. Environmental exposure, estrogen and two X chromosomes are required for disease development in an epigenetic model of lupus. J Autoimmun. 2012;38(2–3):J135–43.
Zandman-Goddard G, Shoenfeld Y. Parasitic infection and autoimmunity. Lupus. 2009;18:1144–8.
Zandman-Goddard G, Solomon M, Barzilai A, Shoenfeld Y. Lupus erythematosus tumidus induced by sex reassignment surgery. J Rheumatol. 2007;34: 1938–40.
Zandman-Goddard G, Berkun Y, Barzilai O, et al. Exposure to Epstein–Barr virus infection is associated with mild systemic lupus erythematosus disease. Ann N Y Acad Sci. 2009;1173:658–63.
Zandman-Goddard G, Solomon M, Rosman Z, Peeva E, Shoenfeld Y. Environment and lupus-related diseases. Lupus. 2012;21:241–50.

Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome)

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Synonyms

Churg-Strauss syndrome; Eosinophilic vasculitis

Definition

Eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome, EGPA) is a systemic small-vessel vasculitis frequently associated with asthma and eosinophilia.

Introduction

EGPA is a systemic disease characterized by asthma, eosinophilia, and vasculitic involvement of various organs or systems, particularly the respiratory tract, the heart, the skin, and the peripheral nervous system. Its main histological features include tissue eosinophilia, necrotizing vasculitis, and eosinophil-rich granulomas (Noth et al. 2003). Given the positivity of antineutrophil cytoplasmic antibodies (ANCA) in a fraction of patients and the predominant involvement of small vessels, EGPA has been grouped under the umbrella term ANCA-associated vasculitis with microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (Wegener's, GPA). The etiopathogenesis of EGPA is still poorly understood, although genetic and environmental factors are likely to play a role. The diagnosis still relies on clinical and histological findings, even if new diagnostic biomarkers are emerging (Zwerina et al. 2011).

Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome), Table 1 Classification criteria and definitions for eosinophilic granulomatosis with polyangiitis (Churg-Strauss)

Lanham et al. 1984
1. Asthma
2. Eosinophilia $>1.5 \times 10^9$/L
3. Clinical or pathological evidence of vasculitis involving at least two organs
American College of Rheumatology 1990[a]
1. Asthma
2. Eosinophilia >10 %
3. Neuropathy (mono- or polyneuropathy)
4. Non-fixed pulmonary infiltrates
5. Paranasal sinus abnormalities
6. Extravascular eosinophil infiltration on biopsy
Chapel Hill Consensus Conference 1994
Eosinophil-rich and granulomatous inflammation involving the respiratory tract, necrotizing vasculitis affecting small to medium-sized vessels, associated with asthma and eosinophilia

[a]At least four of the six American College of Rheumatology criteria are required to classify vasculitis as Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss)

Historical Background, Definition, and Classification Criteria

In 1951, Jacob Churg and Lotte Strauss first described a syndrome characterized by "asthma, fever and eosinophilia, and [. . .]symptoms of cardiac failure, renal damage and peripheral neuropathy resulting from vascular embarrassment in various systems of organs" (Churg and Strauss 1951). The histological examination of the 13 cases (most of which were autopsy cases) described by Churg and Strauss consistently showed the presence of tissue eosinophilia, necrotizing, and granulomatous vascular lesions and extravascular granulomas.

These pathological characteristics still represent the key findings on which the diagnosis of EGPA is based. During the decades that followed the publication of Churg's and Strauss's seminal paper, several attempts were made to establish classification or diagnostic criteria for this syndrome. However, only classification criteria are currently available for EGPA. In 1984, Lanham and colleagues (Lanham et al. 1984) suggested that the presence of asthma, eosinophilia $>1.5 \times 10^9$/L, and vasculitic involvement of two or more organs (with or without histological proof) was a prerequisite for a diagnosis of EGPA (Table 1). However, no validation studies were performed for such criteria. In 1990, the American College of Rheumatology task force for the classification of vasculitis established a set of six criteria (Table 1), four of which were required in order to classify a vasculitis syndrome as EGPA (Masi et al. 1990). In 1994, the Chapel Hill consensus conference nomenclature provided mutually exclusive definitions for the classification of vasculitis and defined EGPA as a eosinophil-rich and granulomatous inflammation involving the respiratory tract, necrotizing vasculitis affecting small to medium-sized vessels, occurring in patients with asthma and eosinophilia (Jennette et al. 1994). In 2012, the revised Chapel Hill classification of vasculitis replaced the traditional term "Churg-Strauss syndrome" with the more descriptive EGPA, but did not provide further criteria for the diagnosis or the classification of the disease (Jennette et al. 2013).

Epidemiology, Genetic, and Environmental Factors

EGPA is a rare disease; its incidence in various populations ranges between 0.5 and 4.2 cases/10^6 persons per year, and its prevalence is 11–14 cases/10^6 persons. EGPA usually arises in people aged 40–60 years, the mean age at diagnosis being approximately 50 years.

No gender predominance, familial clustering, or ethnic predisposition has clearly been shown. However, the influence of genetic factors on disease susceptibility and shaping its clinical phenotype has clearly been demonstrated. Initially shown in an Italian study (Vaglio et al. 2007) and subsequently replicated in a German cohort (Wieczorek et al. 2008a), the HLA-DRB1*04 and *07 alleles and the related HLA-DRB4 gene were found to be associated with an increased susceptibility to develop EGPA; conversely, the HLA-DRB3 was protective, as its frequency was lower in EGPA patients than in healthy controls. Interestingly, the frequency of HLA-DRB4 correlated with the number of vasculitic complications of the disease (e.g., glomerulonephritis, mononeuritis multiplex, purpura) (Vaglio et al. 2007). Of note, another study (Wieczorek et al. 2008b) showed that the ANCA-negative subset of EGPA is associated with the IL10.2 haplotype of the *IL10* gene promoter, which functionally translates into increased IL-10 expression. These data support the hypothesis that the clinically distinct subgroups of EGPA may have different immunogenetic determinants.

Environmental factors have also been thought to contribute to the pathogenesis of EGPA. Occupational exposure to particulate silica has been described as a potential susceptibility factor; additionally, infections and vaccinations have been identified as possible triggers of the disease, but no specific infectious agent or type of vaccination has clearly been implicated. On the other hand, a number of studies have shown that EGPA can be precipitated by the use of certain drugs used in asthmatic patients, in particular leukotriene receptor antagonists (LTRAs) such as montelukast. Different mechanisms have been postulated to explain how LTRAs may trigger EGPA in asthmatic patients: as they may allow steroid tapering, they could unmask smoldering forms of EGPA. Alternatively, they could induce an allergic, idiosyncratic reaction. An additional view is that the development of EGPA during LTRA treatment is simply coincidental and reflects the phasic evolution of the disease through the classical allergic, eosinophilic, and vasculitic stages. The hypothesis linking LTRAs to the unmasking of subclinical disease is the most widely accepted (Hauser et al. 2008) and is also supported by the evidence that other drugs used as steroid-sparing agents in asthma (e.g., theophylline, omalizumab) have been associated with the onset of EGPA.

Pathogenesis

The pathogenetic mechanisms of EGPA are still incompletely understood, but recent studies have shown that different immune cell types are involved in the development of the disease and in mediating tissue damage.

Early reports demonstrated that $CD4^+$ T cells are activated in EGPA and that they undergo oligoclonal expansion; this is in line with the evidence of a restricted HLA repertoire and of the association with HLA class II polymorphisms. $CD4^+$ T-helper 2 (Th2) cell responses are prominent in EGPA. Peripheral $CD4^+$ T-cell lines obtained from the peripheral blood of EGPA patients are able to produce large amounts of Th2 cytokines such as IL4 and IL13, and T cells positive for the Th2 marker CD294 are abundant in EGPA tissue lesions. Additionally, the Th2 cytokine IL5 also seems to be upregulated in active EGPA; IL5 is a major survival factor for eosinophils, as it is able to induce their proliferation, activation, and to prolong their life span. However, Th1 and Th17 responses are also detectable in EGPA; peripheral $CD4^+$ T cells also produce considerable amounts of interferon-γ (IFN γ), a prototypical Th1 cytokine, and Th17 cells – capable of producing IL17A – are found at high frequencies in the peripheral blood of active patients (Vaglio et al. 2012a).

Recent evidence points to B cells and the humoral response as potential contributors to EGPA pathogenesis. The crucial role of B cells has become clear thanks to the use of the B-cell depleting antibody rituximab in several refractory cases (Vaglio et al. 2012a); although no large-scale studies have yet been published, the available reports demonstrate that B-cell depletion may induce not only disease remission but also significant reduction in eosinophil counts and in serum levels of IL5, which is essentially produced by T cells. These findings indicate that B cells may be pivotal in the immune response of EGPA and that they are capable of influencing the function of other cell types such as T cells and eosinophils. The humoral response in EGPA is also likely to be dysregulated. Autoantibodies such as ANCA are detectable in ~40 % of the patients; additionally, IgE levels are commonly high in active EGPA. Finally, a dramatic increase in serum IgG4 levels also characterizes active disease; increased IgG4 production is usually driven by Th2 (IL4, IL13) and regulatory (IL10) cytokines. Notably, IgG4 levels correlate with disease activity and with the number of organs involved (Vaglio et al. 2012b).

Eosinophils are probably the major effector cells in EGPA, as their circulating levels often parallel disease activity, and the release of their granule proteins (e.g., eosinophil basic protein, eosinophil-derived neurotoxin) may induce cell apoptosis and necrosis. Recent evidence suggests that their tissue recruitment is driven by chemokines such as eotaxin-3 (Zwerina et al. 2011), produced by endothelial and epithelial cells. Other chemokines such as CCL17, also produced by "resident" cells such as epithelial cells, can contribute to the amplification of the inflammatory lesions by inducing tissue influx of Th2 cells which, in turn, stimulate eosinophil recruitment and activation (Vaglio et al. 2012a).

Pathology

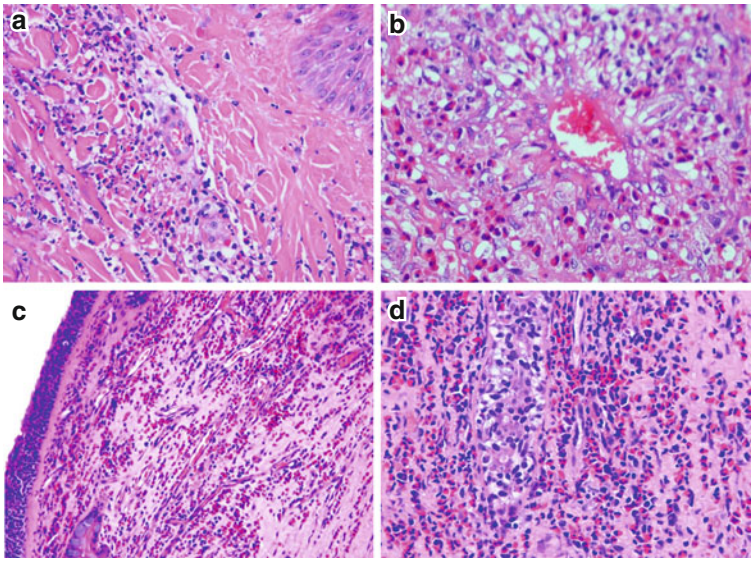
Necrotizing (granulomatous or non-granulomatous) vasculitis, extravascular granulomas, and tissue eosinophilia are the main pathological features of EGPA (Fig. 1). Vasculitis involves the small and, sometimes, medium-sized vessels and is characterized by fibrinoid necrosis and frequently by eosinophilic infiltration. Granulomas may be found within the vascular lesions, but more specific for the diagnosis of EGPA is the extravascular granuloma, which generally consists of a core of amorphous eosinophilic material (probably derived from necrotic eosinophils), surrounded by palisading lymphocytes and epithelioid and multinucleated giant cells (Vaglio et al. 2009).

In EGPA, different disease manifestations may show distinct histopathological patterns. Lung infiltrates usually encompass vasculitis, granulomas, and tissue eosinophilia; cardiomyopathy and gastrointestinal lesions are generally characterized by pronounced eosinophilic infiltration. Conversely, renal lesions usually lack eosinophilic infiltration; likewise, peripheral neuropathy is usually due to epineural vasculitis with lymphomonocytic infiltration of the vasa nervorum but eosinophil infiltration tends to be mild. Purpura and alveolar hemorrhage are also manifestation of capillaritis with few or no eosinophils. Other manifestations, such as sinusitis, bronchial involvement, or skin nodules, usually show only tissue eosinophilia with or without granulomas but lack vasculitis.

Clinical Manifestations

The clinical course of EGPA is usually reported to evolve through an allergic phase, dominated by asthma, rhinosinusitis, and other allergic manifestations; an eosinophilic phase, frequently hallmarked by lung infiltrates, cardiomyopathy, and gastroenteritis; and a vasculitic phase, characterized by organ complications due to small-vessel vasculitis, such as purpura, glomerulonephritis, and peripheral neuropathy. However, these disease phases (especially the eosinophilic and vasculitic phases) often overlap.

Asthma has been reported in 90–100 % of EGPA patients; unlike in usual bronchial asthma, in EGPA it usually arises in adulthood, has no typical seasonal exacerbations, and may precede



Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome), Fig. 1 Representative histological pictures in Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss). (**a**) Skin biopsy showing lymphomonocytic vasculitis with moderate eosinophil infiltration; (**b**) transmural vasculitis of an intestinal submucosa blood vessel showing marked eosinophilic infiltration; (**c**) nasal polyp biopsy showing massive eosinophilic infiltration of the lamina propria with marked oedema; (**d**) biopsy of paranasal sinus mucosa showing pronounced eosinophilic infiltration around a mucin-producing gland of the lamina propria. Original magnification: x20 in **a**, **c** and **d**; x10 in **c**

the full-blown clinical picture of EGPA by many years. Notably, asthma tends to improve when the vasculitis symptoms develop (Vaglio et al. 2009). Asthma is usually accompanied by allergic rhinosinusitis with frequent polyp formation; although the histological appearance of nasal polyps in EGPA is similar to that of typical allergic nasal polyposis, nasal polyps in EGPA tend to recur after surgery if patients do not receive immunosuppression (Bacciu et al. 2008). Unlike GPA, the rhinosinusitis of EGPA does not cause bone erosions of the paranasal and nasal structures, and patients only rarely have nasal crusting or purulent-bloody nasal discharge.

Lung involvement occurs in approximately two thirds of patients with EGPA. Lung infiltrates are usually patchy, peripheral, and sometimes migratory; on high-resolution computed tomography (CT) scanning, they appear as poorly defined areas of consolidation or ground-glass opacities and are often accompanied by abnormalities resulting from bronchial wall involvement, such as bronchial wall thickening, mucous plugs, and small centrilobular nodules. EGPA limited to the respiratory tract must be distinguished from other eosinophilic lung disorders such as acute and chronic eosinophilic pneumonia and allergic bronchopulmonary aspergillosis. Both of these conditions share clinical features with EGPA, including lung infiltrates and an eosinophil-rich bronchoalveolar lavage fluid. However, acute eosinophilic pneumonia is usually a febrile illness with acute onset and sudden respiratory failure. While chronic eosinophilic pneumonia tends to begin more insidiously, both the acute and chronic forms of eosinophilic pneumonia usually lack other organ manifestations and the ANCA is usually negative. Allergic pulmonary aspergillosis may mimic EGPA forms limited to the airway tract also because it causes bronchial involvement; the isolation of Aspergillus species in the sputum or bronchoalveolar lavage fluid and the presence of Aspergillus-specific IgE in the serum are distinguishing features of this condition. Other lung manifestations in EGPA include pleural

effusion due to pleuritis or cardiomyopathy-related congestive heart failure and, rarely, alveolar hemorrhage.

Cardiac involvement is also frequent, especially if patients are screened with cardiac magnetic resonance imaging or echocardiography. Endomyocarditis is the most common pattern of cardiac involvement, but pericarditis and coronary vasculitis may also occur. EGPA-related endomyocarditis may lead to restrictive cardiomyopathy and chronic heart failure. In the eosinophilic phase of the disease, gastrointestinal involvement is also frequent; it usually presents as abdominal pain and can be complicated by gastrointestinal bleeding. The stomach and small bowel are more frequently affected, although there are some reports of involvement of the large bowel and cholecystitis. Heart and gastrointestinal involvement are predictors of poor prognosis in EGPA (Noth et al 2003).

Constitutional symptoms such as weight loss, anorexia, fatigue, and fever often herald the vasculitic or generalized phase of EGPA. Peripheral neuropathy is a cardinal feature of this disease stage, as it occurs in ~70 % of the cases; it can cause sensory and/or motor deficits and, in some cases, neuropathic pain, and more frequently involves the peroneal, tibial, ulnar, and median nerves. It is often asymmetrical, with a pattern defined as mononeuritis multiplex, and on electrophysiological studies it shows signs of axonal damage. Although rare, central nervous system (CNS) involvement may cause stroke, cranial nerve palsies, or cognitive disturbance (among other symptoms) and represents a major adverse prognostic factor.

Skin lesions, particularly purpura, also result from small-vessel vasculitis. Purpura has been reported in ~25 % of patients, and similar to that observed in other systemic vasculitis syndromes such as ANCA-associated and cryoglobulinemic vasculitis, it predominantly affects the lower limbs. Other dermatological manifestations include nodules, urticaria, livedo reticularis, and ulcers.

Renal abnormalities are found in ~25 % of the cases and range from isolated urinary abnormalities such as proteinuria and microhematuria to rapidly progressive glomerulonephritis. The histological picture is similar to that of the other ANCA-associated vasculitides, with findings of necrotizing, crescentic (i.e., with extra-capillary proliferation leading to the formation of crescents) glomerulonephritis with few or no immune deposits ("pauci-immune"). However, both histological lesions and renal manifestations in EGPA are usually much less severe than those found in GPA or MPA, and patients only rarely progress to renal failure (Sinico et al 2006). Nevertheless, proteinuria >1 g/day and serum creatinine >140 μmol/L are predictors of poor outcome in EGPA.

Laboratory Findings

Significant peripheral eosinophilia (usually >1,500 cells/μL or >10 %) is nearly always present in patients with active EGPA, except when masked by steroid therapy. Eosinophilia frequently correlates with disease activity; the reappearance of eosinophilia during remission may be an early sign of relapse. Erythrocyte sedimentation rate and/or C-reactive protein levels may also be high during active disease, although they often do not mirror disease activity. Serum IgE are high in ~80 % of the cases, but they lack specificity for common allergens. Among IgG subclasses, a dramatic increase in IgG4 levels has been found in patients with active disease. Again, the specificity of such immunoglobulins has not yet been investigated (Vaglio et al. 2012b).

ANCA always need to be tested in patients with suspected EGPA; they are positive in 35–40 % of the cases, and their immunofluorescence pattern is perinuclear (P-ANCA) in 74–90 % and cytoplasmic (C-ANCA) or mixed in the rest. P-ANCA generally correspond (using ELISA) to anti-myeloperoxidase (MPO) antibodies, while the C-ANCA pattern is generally the result of anti-proteinase 3 (PR3) antibodies; a positive ANCA in a high titer (especially when due to anti- MPO or anti-PR3 antibodies) is useful in the differential diagnosis

between EGPA and different eosinophilic conditions, including the so-called hypereosinophilic syndromes (HESs). Although no systematic studies have been conducted, it is generally accepted that most variants of the HES (including those related to chromosomal abnormalities such as the *FIP1L1-PDGFRA* fusion gene, the lymphocytic variant, chronic eosinophilic leukemia, and idiopathic HES) are ANCA-negative. However, since most EGPA patients are also ANCA-negative, the differential diagnosis between EGPA and HES is often challenging; testing serum levels of different cytokines and chemokines (e.g., sIL2R, IL5, IL6, IL8, IL10, CCL17, eotaxin-1) failed to differentiate ANCA-negative EGPA and idiopathic HES (Khoury et al. 2012). In a recent study, however, serum eotaxin-3, a eosinophil-attracting chemokine, proved a reliable diagnostic marker of active EGPA, with control groups including not only HES but also other vasculitic syndromes, secondary forms of eosinophilia, and systemic connective tissue disorders (Zwerina et al. 2011).

In EGPA, ANCAs also have a role in distinguishing clinical phenotypes of the disease. Specifically, ANCA-positive patients more frequently have peripheral neuropathy, glomerulonephritis, or purpura (which are due to small-vessel vasculitis), whereas endomyocardial involvement and lung infiltrates prevail in the ANCA-negative subset. In addition, signs of vasculitis on biopsy tend to be more frequent in ANCA-positive cases. Based on these findings, it has been hypothesized that two major subsets of EGPA exist, one ANCA-positive, in which tissue damage is mainly mediated by vasculitis, and one ANCA-negative, dominated by organ manifestations related to eosinophil tissue infiltration. This distinction is not clear-cut, however, and manifestations of the two subsets often overlap (Sinico et al. 2005; Sablé-Fourtassou et al. 2005).

Treatment and Outcome

Different immunosuppressive regimens have been proposed for the treatment of EGPA, but there is at present no consensus regarding a staged, remission-induction, and remission-maintenance approach. The prognostic profile of EGPA patients may influence therapeutic decisions. The five-factor score (FFS), the most used prognostic score for EGPA, includes five poor prognosis factors, namely, heart, gastrointestinal, and CNS involvement, creatinine >140 μmol/L, and proteinuria >1 g/24 h. Patients with one or more of these five factors are usually treated with combinations of glucocorticoids and immunosuppressants (generally cyclophosphamide for induction of remission), whereas those with a FFS $= 0$ are initially treated with glucocorticoids alone (Cohen et al. 2007). However, combinations of glucocorticoids and immunosuppressants are commonly used also for patients who have a FFS $= 0$ but bear other severe disease manifestations such as alveolitis or polyneuropathy.

In a trial of patients with no poor prognosis factors, remission was achieved using glucocorticoids alone in 90 % of patients; however, during the follow-up, 35 % of the patients relapsed and eventually required combination therapies based on azathioprine or methotrexate plus glucocorticoids (Ribi et al. 2008). In a trial of EGPA patients with FFS ≥ 1, cyclophosphamide plus prednisone was used as induction therapy, and patients were randomized to receive either 6 or 12 monthly cyclophosphamide pulses. The two regimens had similar efficacy in inducing remission, but relapses (especially minor) were more frequent in the 6-pulse group (Cohen et al. 2007).

Other traditional immunosuppressive drugs used for remission induction include methotrexate and intravenous immunoglobulins (IVIg). Methotrexate proved effective in remission induction in combination with prednisone, but relapses were frequent ($\sim$50 %) during the maintenance phase. IVIg was used in a small pilot trial together with plasma exchange, cyclophosphamide, and glucocorticoids, with good short- and long-term results (Danieli et al. 2004); however, no further trials have explored their efficacy and safety.

The duration of therapy is a matter of debate, but it is usually accepted that combination

therapies should be continued for at least 12 months. Many patients are continued even longer on low-dose glucocorticoids, which are often needed to control residual asthma.

EGPA may prove refractory to standard immunosuppressive therapy or dependent on high-dose glucocorticoids; in addition, many patients have significant toxicity or contraindications to high-dose glucocorticoids. For such cases, alternative treatments have been explored. Interferon-α, due to its ability to impair eosinophil degranulation and Th2 responses in vitro, has been used in refractory EGPA cases with positive results (Tatsis et al. 1998); however, most patients who were kept on this treatment for remission maintenance developed relapses or had to discontinue treatment because of toxicity. Recent pilot trials (Kim et al. 2010; Moosig et al. 2011) have demonstrated the efficacy of mepolizumab, a monoclonal antibody that targets IL5, the major survival factor for eosinophils. Mepolizumab was used both in refractory and glucocorticoid-dependent patients. Although it allowed significant reduction in glucocorticoid dose, relapses occurred in almost all cases upon its cessation (Moosig et al. 2011). Numerous case reports or small case series have described that rituximab, a B-cell depleting monoclonal antibody, may also be effective in refractory EGPA. In most cases rituximab induced not only disease remission but also a drop in eosinophil counts and acute-phase reactants (Cartin-Ceba et al. 2011).

The outcome of EGPA is generally good: survival rates at 1 and 5 years may be as high as 100 % and 97 %; in the trial of patients with poor-prognosis factors, 92 % were alive at the 8-year follow-up analysis. However, disease-related organ damage such as heart failure and chronic neuropathy may severely impair the quality of life of patients with EGPA. Immunosuppressive treatment can also contribute to morbidity, including an increased risk of malignancy and infection. In particular, the long-term use of glucocorticoids is often the cause of chronic, debilitating conditions such as diabetes and osteoporosis.

Cross-References

- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Vasculitis and the Kidney
- ▶ Vasculitis: Granulomatosis with Polyangiitis (Wegener's)

References

Bacciu A, Buzio C, Giordano D, Pasanisi E, Vincenti V, Mercante G, et al. Nasal polyposis in Churg-Strauss syndrome. Laryngoscope. 2008;118(2):325–9.

Cartin-Ceba R, Keogh KA, Specks U, Sethi S, Fervenza FC. Rituximab for the treatment of Churg-Strauss syndrome with renal involvement. Nephrol Dial Transplant. 2011;26(9):2865–71.

Churg J, Strauss L. Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. Am J Pathol. 1951;27(2):277–301.

Cohen P, Pagnoux C, Mahr A, Arene JP, Mouthon L, Le Guern V, et al. Churg-Strauss syndrome with poor-prognosis factors: a prospective multicenter trial comparing glucocorticoids and six or twelve cyclophosphamide pulses in forty-eight patients. Arthritis Rheum. 2007;57(4):686–93.

Danieli MG, Cappelli M, Malcangi G, Logullo F, Salvi A, Danieli G. Long term effectiveness of intravenous immunoglobulin in Churg-Strauss syndrome. Ann Rheum Dis. 2004;63(12):1649–54.

Hauser T, Mahr A, Metzler C, Coste J, Sommerstein R, Gross WL, et al. The leucotriene receptor antagonist montelukast and the risk of Churg-Strauss syndrome: a case-crossover study. Thorax. 2008;63(8):677–82.

Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum. 1994;37(2):187–92.

Jennette J, Falk R, Bacon P, Basu N, Cid M, Ferrario F, et al. 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides. Arthritis Rheum. 2013;65(1):1–11.

Khoury P, Zagallo P, Talar-Williams C, Santos CS, Dinerman E, Holland NC, et al. Serum biomarkers are similar in Churg-Strauss syndrome and hypereosinophilic syndrome. Allergy. 2012;67(9):1149–56.

Kim S, Marigowda G, Oren E, Israel E, Wechsler ME. Mepolizumab as a steroid-sparing treatment option in patients with Churg-Strauss syndrome. J Allergy Clin Immunol. 2010;125(6):1336–43.

Lanham JG, Elkon KB, Pusey CD, Hughes GR. Systemic vasculitis with asthma and eosinophilia: a clinical approach to the Churg-Strauss syndrome. Medicine (Baltimore). 1984;63(2):65–81.

Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, et al. The American College of

Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum. 1990;33(8):1094–100.
Moosig F, Gross WL, Herrmann K, Bremer JP, Hellmich B. Targeting interleukin-5 in refractory and relapsing Churg-Strauss syndrome. Ann Intern Med. 2011;155(5):341–3.
Noth I, Strek ME, Leff AR. Churg-Strauss syndrome. Lancet. 2003;361(9357):587–94.
Ribi C, Cohen P, Pagnoux C, Mahr A, Arene JP, Lauque D, et al. Treatment of Churg-Strauss syndrome without poor-prognosis factors: a multicenter, prospective, randomized, open-label study of seventy-two patients. Arthritis Rheum. 2008;58(2):586–94.
Sablé-Fourtassou R, Cohen P, Mahr A, Pagnoux C, Mouthon L, Jayne D, et al. Antineutrophil cytoplasmic antibodies and the Churg-Strauss syndrome. Ann Intern Med. 2005;143(9):632–8.
Sinico RA, Di Toma L, Maggiore U, Bottero P, Radice A, Tosoni C, et al. Prevalence and clinical significance of antineutrophil cytoplasmic antibodies in Churg-Strauss syndrome. Arthritis Rheum. 2005;52(9):2926–35.
Sinico RA, Di Toma L, Maggiore U, Tosoni C, Bottero P, Sabadini E, et al. Renal involvement in Churg-Strauss syndrome. Am J Kidney Dis. 2006;47(5):770–9.
Tatsis E, Schnabel A, Gross WL. Interferon-alpha treatment of four patients with the Churg-Strauss syndrome. Ann Intern Med. 1998;129(5):370–4.
Vaglio A, Martorana D, Maggiore U, Grasselli C, Zanetti A, Pesci A, et al. HLA-DRB4 as a genetic risk factor for Churg-Strauss syndrome. Arthritis Rheum. 2007;56(9):3159–66.
Vaglio A, Casazza I, Grasselli C, Corradi D, Sinico RA, Buzio C. Churg-Strauss syndrome. Kidney Int. 2009;76(9):1006–11.
Vaglio A, Moosig F, Zwerina J. Churg-Strauss syndrome: update on pathophysiology and treatment. Curr Opin Rheumatol. 2012a;24(1):24–30.
Vaglio A, Strehl JD, Manger B, Maritati F, Alberici F, Beyer C, et al. IgG4 immune response in Churg-Strauss syndrome. Ann Rheum Dis. 2012b;71(3):390–3.
Wieczorek S, Hellmich B, Gross WL, Epplen JT. Associations of Churg-Strauss syndrome with the HLA-DRB1 locus, and relationship to the genetics of antineutrophil cytoplasmic antibody-associated vasculitides: comment on the article by Vaglio et al. Arthritis Rheum. 2008a;58(1):329–30.
Wieczorek S, Hellmich B, Arning L, Moosig F, Lamprecht P, Gross WL, et al. Functionally relevant variations of the interleukin-10 gene associated with antineutrophil cytoplasmic antibody-negative Churg-Strauss syndrome, but not with Wegener's granulomatosis. Arthritis Rheum. 2008b;58(6):1839–48.
Zwerina J, Bach C, Martorana D, Jatzwauk M, Hegasy G, Moosig F, et al. Eotaxin-3 in Churg-Strauss syndrome: a clinical and immunogenetic study. Rheumatology (Oxford). 2011;50(10):1823–7.

Epigenetics in Autoimmunity

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Synonyms

Chromatin remodeling

Definition

The term *Epigenetics* summarizes a group of regulatory mechanisms that affect gene expression. *Epigenetics* originates from the Greek words *επί* (*over*) and *γενετικός* (*genetics*). Epigenetic patterns of genes determine their signal- and tissue-specific expression without altering the DNA sequence. Epigenetic effects on gene expression are mediated by the reorganization of nucleosomes and chromatin fibers which affect the recruitment of transcription factors and RNA polymerases to genes. Thus, epigenetic patterns determine gene expression and are essential during cell and tissue development and differentiation. Epigenetic marks are generally variable, allowing cells and tissues to adapt to environmental changes (Brooks et al. 2010; Grolleau-Julius et al. 2010; Meda et al. 2011; Hedrich et al. 2011; Renaudineau and Youinou 2011).

Epigenetic Mechanisms in Gene Regulation

In order to exert their biological functions, transcription factors bind to their corresponding *cis*-DNA sequences within the regulatory regions

of genes. An accessible DNA structure is required for the binding of transcription factors to these regions, and an efficient way of silencing a gene is through the prevention of transcription factor binding.

CpG-DNA methylation: One way of preventing transcription factor binding is the addition of methyl groups to the 5′ carbon position of cytosine within cytosine-phosphate-guanosine (CpG-)dinucleotides. This is mediated through *DNA methyltransferases* (*DNMTs*) and can occur during cell division (DNMT1 re-methylates hemi-methylated CpGs, maintaining CpG-DNA methylation patterns) or independent of the cell cycle in the context of *de novo* methylation (DNMT3a and DNMT3b). Attenuated CpG-DNA methylation has been linked to the development of multiple diseases, including autoimmune disorders, infectious diseases, and cancer (Hedrich and Tsokos 2011; Renaudineau and Youinou 2011).

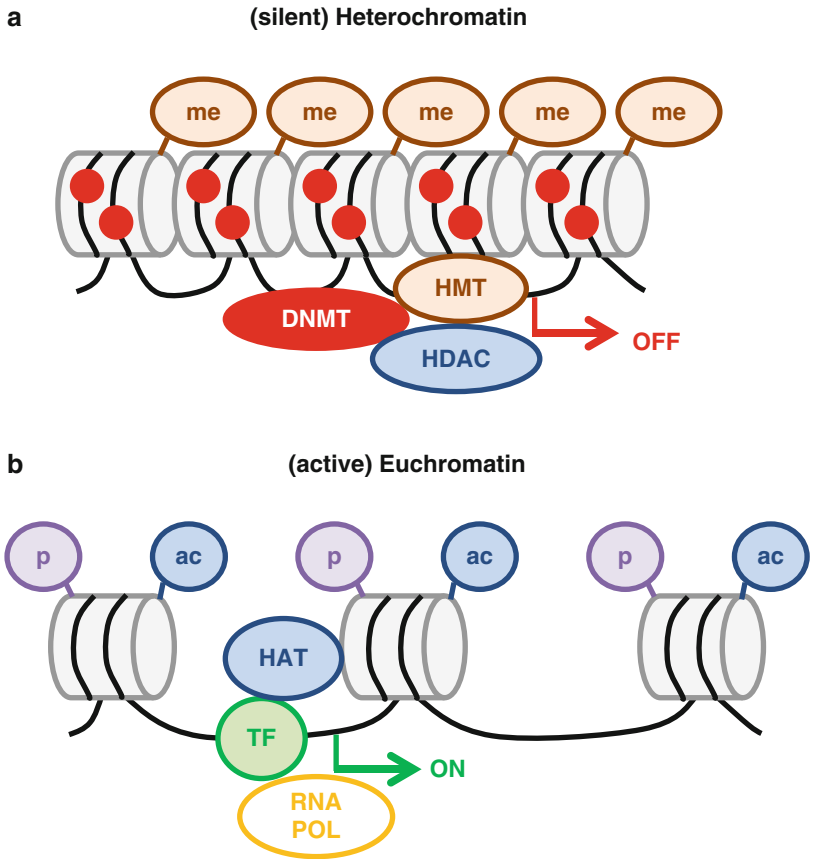
Histone modifications: Nucleosomes are the basic subunit of chromatin. A nucleosome is comprised of 146 base pairs of genomic DNA coiled around a histone octamer consisting of two copies of each of the histone proteins: H2A, H2B, H3, and H4. All histone proteins contain flexible N-terminal tails that undergo posttranslational modifications. Posttranslational histone modifications strongly impact the organization of nucleosomes, thus affecting the accessibility of DNA to transcription factors and RNA polymerases. Histone modifications include acetylation, methylation, ubiquitination, phosphorylation, sumoylation, citrullination, ADP ribosylation, and proline isomerization. Single markers and the combination of various histone modifications facilitate changes in the arrangement of nucleosomes and the resulting chromatin structure (Fig. 1) (Brooks et al. 2010; Grolleau-Julius et al. 2010; Meda et al. 2011; Hedrich and Tsokos 2011; Renaudineau and Youinou 2011).

CpG-DNA methylation and histone modifications usually follow corresponding patterns. Transcriptionally inactive (hetero-)chromatin is characterized by a high degree of CpG-DNA methylation and inactivating histone modifications, such as histone H3 lysine 9 (H3K9) and/or H3K27 tri-methylation. Active genes exhibit low levels of CpG-DNA and histone methylation, but high levels of histone acetylation (e.g., H3K9ac and H3K18ac) (Wilson et al. 2005; Brenner and Fuks 2007).

Micro RNAs (miRNAs): MiRNAs are small RNAs spanning 21 to 23 base pairs. They function as posttranscriptional regulators of gene expression. MiRNAs have been categorized as one of the three central epigenetic mechanisms in gene regulation, and miRNAs are in close relation to CpG-DNA methylation and histone modifications (Fig. 2). MiRNAs derive from larger intergenic and intronic transcripts. There is a tight correlation between the presence of intergenic transcripts and gene expression. Thus, intergenic transcription has been suggested to be at the interface of chromatin remodeling and the transcription of genes, allowing for interactions between distal regulatory elements and core promoters. RNA polymerases II or III transcribe so-called preliminary miRNAs. These preliminary RNAs are subsequently cleaved by the ribonuclease Drosha before they are exported to the cytoplasm. Dicer further processes the transcripts into mature miRNAs which form complexes with mRNA from the 3′ UTR of target genes. These interactions result in translational repression through direct mRNA cleavage or translational arrest (Thai et al. 2010; Hedrich and Tsokos 2011; Meda et al. 2011).

Immune homeostasis is maintained by a tight balance between activating and inactivating regulatory mechanisms. Changes in the epigenetic marks of single or multiple cells or tissues can contribute to autoimmune pathology on multiple levels.

Epigenetic mechanisms are involved in (1) the regulation of multiple genes, including genes encoding for cytokines, (co-)stimulatory molecules, and transcription factors, and (2) cell and tissue differentiation and lineage commitment. Furthermore, histone modifications have been documented to function as strong autoantigens and contribute to antibody production in systemic lupus erythematosus (SLE) and rheumatoid arthritis.



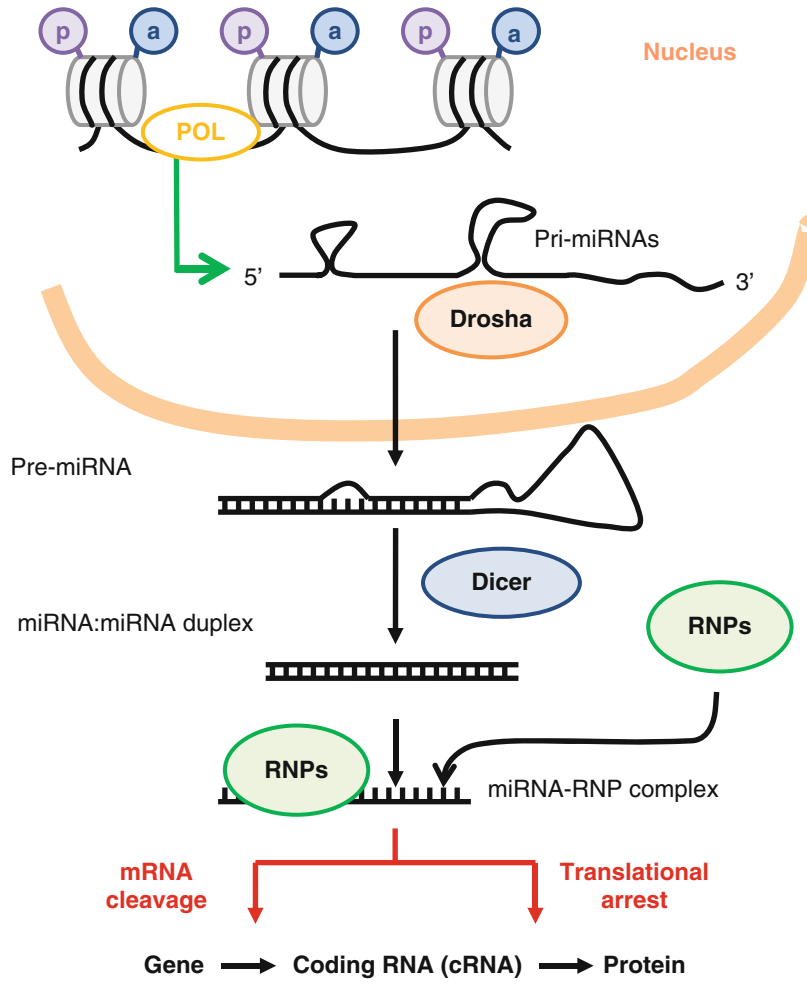
Epigenetics in Autoimmunity, Fig. 1 Nucleosome arrangement and chromatin structure in silent, transcriptionally inactive heterochromatin (**a**) and active euchromatin (**b**). Compact heterochromatin is characterized by dense packing of nucleosomes, repressive histone modifications (*brown circles*, representing histone hypermethylation), and high levels of CpG-DNA methylation (*red circles*). In these regions, recruitment of DNA methyltransferases (DNMTs), histone methyl transferases (HMTs), and histone deacetylases (HDACs) mediates a "closed" chromatin conformation. Active euchromatin is characterized by permissive histone modifications (*purple circles*, representing histone phosphorylation; *blue circles*, representing histone acetylation) and low levels of CpG-DNA methylation mediating decompaction. In these regions, transcription factors can bind to DNA motifs and recruit RNA polymerases (*yellow circle*) and additional activating epigenetic modifiers (HAT: histone acetyl transferases, blue circle) (Brenner and Fuks 2007; Hedrich et al. 2010; Wilson et al. 2005)

In the following, epigenetic mechanisms contributing to inflammation and antibody production in autoimmune diseases will be discussed.

Attenuated Epigenetic Control and Autoimmunity

Over the past years, the influence of epigenetic mechanisms on autoimmune diseases has been documented in various studies. It appears likely that epigenetic mechanisms may bridge the gap between genomic predispositions for the development of a disease and environmental factors that contribute to the pathogenesis of autoimmune disorders.

A number of immunological pathways have been demonstrated to be controlled or influenced by epigenetic mechanisms. Epigenetic mechanisms play a central role in the regulation of lymphocyte differentiation. The expression of the lineage determining *Il4* and *IFNg* genes in



Epigenetics in Autoimmunity, Fig. 2 MiRNAs are derived from noncoding transcripts of regions with "open" chromatin that are accessible to RNA polymerases (*yellow circle*). Primary transcripts (Pri-miRNAs) are processed into preliminary (pre-)miRNAs by the ribonuclease Drosha. Pre-miRNAs are transported to the cytoplasm and processed into mature miRNAs by Dicer. Mature miRNAs get incorporated into ribonuclease (RNP) complexes that exert their regulatory functions: translational arrest and mRNA cleavage (Brenner and Fuks 2007; Hedrich et al. 2010; Thai et al. 2010; Wilson et al. 2005)

T lymphocytes has been demonstrated to be under tight epigenetic control (Schoenborn et al. 2007). While the genes of naïve $CD4^+$ T lymphocytes are partially silenced, *Il4* is silenced in Th1 and *IFNg* is silenced in Th2 subsets. Similarly, the IL-10-related cytokine IL-19 is silenced in T lymphocytes through CpG-DNA methylation, while IL-19-expressing macrophages display site- and tissue-specific demethylation of several regions of the *Il19* gene (Hofmann et al. 2012). It seems likely that more genes with relevance to lymphocyte development and differentiation are under epigenetic control.

Epigenetic alterations of cytokine genes have been associated to the expression of autoimmune disease. In SLE, general ***hypomethylation of CpG-DNA*** correlates with disease activity, and several methylation-sensitive genes are over-expressed in $CD4^+$ T lymphocytes from SLE patients, including *IL4*, *IL6*, *IL10*, *IL13*, and *IL17* (Hedrich and Tsokos 2011; Rauen et al. 2011). Furthermore, a number of signaling molecules are subject to CpG-DNA hypomethylation in SLE T and B lymphocytes: *CD70/CD26L*, *CD6*, *CD11*, *CD40L/CD154* (in T cells), and *CD5* (in B cells) are over-expressed as a result of CpG-DNA hypomethylation. Interestingly, the *IL2* gene that fails to be expressed in SLE T lymphocytes is subject to epigenetic silencing through CpG-DNA and histone methylation (Renaudineau and Youinou 2011; Hedrich and Tsokos 2011). Similarly to SLE lymphocytes, rheumatoid arthritis synovial fibroblasts exhibit a hypomethylated state. Demethylated

CpG-elements within the *IL6* promoter have been demonstrated to be responsible for monocyte activation and inflammation. Hypomethylation and subsequent activation of LINE-1, a human endogenous retroviral element, has been demonstrated to contribute to the autoimmune phenotype of rheumatoid arthritis (Neidhart et al. 2000; Nile et al. 2008) (Table 1).

Histone modifications also play a role in the pathophysiology of autoimmune disorders. Thus far, histone modifications reported in SLE have been complex. Tissue-specific histone acetylation in some regions has been associated with increased disease activity, whereas histone acetylation in other regions seems to have protective effects. Increased acetylation of the *TNFα* promoter in monocytes from SLE patients is associated with increased monocyte maturation and cytokine expression (Sullivan et al. 2007; Hedrich and Tsokos 2011). Contrarily, histone acetylation of the *IL2* promoter is decreased and histone methylation is increased in T lymphocytes from SLE patients (Hedrich and Tsokos 2011). Fibroblasts from patients with rheumatoid arthritis exhibit global hyperacetylation of histone proteins. Monocytes from patients with chronic recurrent multifocal osteomyelitis fail to produce IL-10. This is associated with attenuated recruitment of the transcription factor Sp1 to the *IL10* promoter and reduced histone phosphorylation (Hofmann et al. 2011). Taken together, the involvement of histone modifications in the pathogenesis of autoimmune disorders appears certain. However, patterns are complex with areas of tissue-specific hyper- and hypo-acetylation (Table 1).

The involvement of ***miRNAs*** in various regulatory mechanisms during cell differentiation and in immune regulation embodies a huge potential for dysregulation and disease expression. However, the number of miRNAs documented to be involved in the pathogenesis of SLE and other autoimmune disorders is rather limited. The groups involved in the expression of SLE are mainly involved in the regulation of innate inflammatory responses by abnormal activation of the type I interferon pathway (miR-146a); inflammatory responses by suppressing the transcription

Epigenetics in Autoimmunity, Table 1 Selection of central epigenetic modifications in autoimmune disorders

CpG-DNA methylation	
Reduced CpG-DNA methylation	
SLE	Global hypomethylation of DNA in B and T lymphocytes
	In vivo and in vitro CpG-DNA demethylation results in SLE-like symptoms
	Altered DNA methyltransferase expression in SLE T lymphocytes
	CpG-DNA demethylation in SLE is mediated by GADD45a
	Hypomethylation and increased expression of cytokine genes: *IL4*, *IL6*, *IL10*, *IL13*, *IL17*
	Demethylation of co-stimulatory molecules: *CD6*, *CD11A*, *CD40L*, *CD70*, *CD5*
	Demethylation of perforin-1 (*PRF1*) mediates lysis of monocytes
	Demethylation and increased expression of the protein phosphatase 2A (*PP2A*)
	Demethylated DNA induces anti-DNA antibody production
	Demethylation of the human endogenous retroviral element (HERV) LINE-1
	. . . etc.
RA	Hypomethylation and increased expression of *IL6*
	In vitro demethylation of fibroblasts results in a RA-like phenotype
	Demethylation of the human endogenous retroviral element (HERV) LINE-1
MS	CpG-DNA demethylation in inflammatory white matter lesions
	Demethylation of the peptidylarginine deaminase 2 (*PAD2*) promoter:
	Attenuated proteolytic digestion of myelin and myelin instability
	Enhanced T cell responses
Increased CpG-DNA methylation	
SLE	Hypermethylation of cytokine genes in SLE T lymphocytes : *IL2*
RA	Increased methylation of the death receptor 3 (*DR3*) gene
SSc	Increased CpG-DNA methylation of the *FLI* promoter
T1D	Increased CpG-DNA methylation:
	Impaired homocysteine metabolism and tissue damage
	Impaired lymphocyte function and inflammation
Histone modifications	
Increased histone acetylation	
SLE	Globally increased histone acetylation
	Tumor necrosis factor (*TNF*) promoter:
	Increased TNF-α expression
	Increased monocyte maturation

(*continued*)

Epigenetics in Autoimmunity, Table 1 (continued)

RA	Globally increased histone acetylation
SSc	Histone deacetylase inhibitors mediate increased FL1 expression and fibrosis
	Reduced histone acetylation
SLE	Reduced acetylation of the *IL2* promoter in SLE T lymphocytes
	Increased histone deacetylase 1 (HDAC1) recruitment to the *IL2* promoter in SLE T cells
	Lupus-prone MRL/lpr mice express the histone deacetylase sirtuin-1 at increased levels
	Lupus-prone MRL/lpr mice express histone acetyltransferases p300, p300/CBP-associated factor, and HDAC7 at reduced levels
RA	Histone deacetylase inhibitors improve symptoms in murine RA models
	Histone modifications and autoantibody production
SLE	Apoptosis-related histone modifications enhance antibody production
	SLE autoantibodies react to apoptosis-related histone modifications
miRNAs	
SLE	miRNA-17 ~ 92: SLE-like symptoms in mouse model
	miRNA-125a: suppression of KLF13 and RANTES results in inflammation
	miRNA-126: interacts with DNMT1 and mediates *CD70* demethylation
	miRNA-146a: activation of type 1 interferon pathways
	miRNA-148a: direct and indirect targeting of DNMT1
	miRNA-148a: direct and indirect targeting of DNMT1
RA	miRNA-146a: activation of type 1 interferon pathways
	miRNA-155: suppression of metalloproteases in RA synovial fibroblasts
	miRNA-203: upregulated in RA synovial fibroblasts, resulting in increased IL-6 and matrix metalloprotease 1 expression
SjS	miRNA-150: upregulated in salivary glands of diabetic mice with SjS
	miRNA-574-3p and miRNA-768-3p: over-expressed in salivary glands

SLE systemic lupus erythematosus, *RA* rheumatoid arthritis, *T1D* type 1 diabetes, *MS* multiple sclerosis, *SSc* systemic sclerosis, *SjS* Sjögren's syndrome
Brooks et al. (2010), Grolleau-Julius et al. (2010), Meda et al. (2011), Hedrich and Tsokos (2011), Renaudineau and Youinou (2011), Thai et al. (2010), Hofmann et al. (2011), Rauen et al. (2011), Neidhart et al. (2000), Nile et al. (2008), Sullivan et al. (2007), Stanczyk et al. (2008), Chuang and Jones (2007), Zhao et al. (2010), Fraga et al. (2005)

factors KLF13 and RANTES (miR-125a); and CpG-DNA demethylation (miR-21, miR148a). MiR-146, miR-574, and miR-768-3p have been shown to be expressed in salivary glands and peripheral tissues from patients with Sjögren's syndrome (Brooks et al. 2010; Hedrich and Tsokos 2011). Mice that develop a rheumatoid arthritis–like syndrome exhibit miR-146a, and miR-155 expression in synovial fibroblasts. Furthermore, miR-203 was upregulated in these cells in a CpG-DNA methylation-dependent manner, resulting in increased IL-6 and matrix metalloproteinase1 expression (Stanczyk et al. 2008; Nakasa et al. 2008) (Table 1).

A strong interplay between miRNAs and other epigenetic mechanisms has been documented recently. Several of the genes regulated by mRNAs are involved in the epigenetic regulation of cellular functions. MiRNAs can influence CpG-DNA methylation through variable DNMT3a and DNMT3b expression, and DNMT1a transcript stability (Chuang and Jones 2007; Hedrich and Tsokos 2011). Overexpression of miR-126 in $CD4^+$ T lymphocytes results in the demethylation of *CD11A* and *CD70*, resulting in increased B and T lymphocyte activation (Zhao et al. 2010).

X Chromosomal Inactivation and Autoimmune Disease

The fact that females have two X chromosomes while males only have one raises the question of whether gender contributes to the female predominance in SLE (10:1), primary Sjögren's syndrome (9:1), rheumatoid arthritis (3:1), multiple sclerosis (2:1), and other autoimmune disorders (Brooks et al. 2010). The X and the Y chromosomes are believed to be derived from a common ancestor autosome. They still comprise similar genes in their pseudo-autosomal regions. Since most X-linked genes are not gender specific, they exhibit equal expression patterns in females and males. To achieve this, female mammalian organisms inactivate one X chromosome in a complicated epigenetic event, called X chromosome inactivation. X chromosome

inactivation involves all levels of epigenetic mechanisms, including CpG-DNA methylation, inactivating histone modifications (namely H3K9me2, H3K27me3, H3K20me, and reduced acetylation of H4), and miRNAs. Under physiological conditions, approximately 35 % of all genes on the short arms and 5 % of the genes on the long arms of the inactivated X chromosome are partially active. Many of these genes are also active on the Y chromosome and do not require compensation. Genes that require transcriptional control in order to avoid unbalanced expression can contribute to disease pathogenesis. This can be mediated through an abnormal number of X chromosomes (Klinefelter's syndrome: 47, XXY; Turner's syndrome: 45, X0) or incomplete X chromosomal silencing, resulting in over-expression of single genes (Brooks et al. 2010; Hedrich and Tsokos 2011).

A number of X chromosomal anomalies have been suggested to affect autoimmune disorders. Women with a complete chromosomal set of 46, XX and men with an additional X chromosome (47, XXY) develop SLE more frequently than healthy men (46, XY) (ratio 10:1). Also, an impaired X chromosomal inactivation has been documented to be associated with autoimmune disorders, including systemic sclerosis, autoimmune thyroiditis, Sjogren's syndrome, and rheumatoid arthritis. Women that lack one X chromosome (Turner's syndrome; 45, X0) develop SLE incidences that are comparable to those in men. Interestingly, fragmentation or absence of one X chromosome seems to increase the risk to develop other autoimmune disorders, such as type 1 diabetes. The loss of one X chromosome in peripheral cells, for example, is associated with primary biliary cirrhosis, autoimmune thyroiditis, but not associated with SLE (Brooks et al. 2010; Renaudineau and Youinou 2011; Hedrich and Tsokos 2011).

Age and Autoimmunity

A constantly increasing body of literature supports the hypothesis that epigenetic dysregulation contributes to the growing incidence of autoimmune disease and cancer with age (Grolleau-Julius et al. 2010), thus providing a mechanistic link between immune-senescence and autoimmune disorders. A progressive reduction of CpG-DNA methylation has been documented with growing age that appears to be proportional to life expectancy. Age-related epigenetic changes frequently accumulate in regions that contain homeobox genes (which are involved in the regulation of morphogenesis) and genes that are involved in cell differentiation (Brooks et al. 2009). Hypermethylation of tumor suppressor genes, aberrant DNMT activity that can result in loss of genomic imprinting, and chromosomal translocations in hypomethylated CpG-DNA sequences contribute to imbalances of the immune homeostasis in the elderly (Grolleau-Julius et al. 2009; Bollati et al. 2009; Brooks et al. 2009). It has been demonstrated that CpG-DNA methylation and histone H3 and H4 acetylation in PBMCs from elderly monozygotic twins diverge from younger controls, suggesting that these epigenetic variants accumulate with age (Fraga et al. 2005). In addition to age, epigenetic patterns can change in response to environmental exposure, explaining inter-individual differences in epigenetic patterns between monozygotic twins (Grolleau-Julius et al. 2010).

Recent evidence indicates the contribution of age-related changes in miRNA expression to autoimmunity and cancer (Grolleau-Julius et al. 2010). This is of special interest, since miRNAs on the one hand originate from "open" chromatin that has low degrees of CpG-DNA methylation, but can on the other hand also influence the degree of CpG-DNA methylation. Thus, it has been suggested that the involvement of the aforementioned miR-146 and miR-155 in rheumatoid arthritis is age dependent and may be indicative for further age-related disorders (Stanczyk et al. 2008).

Epigenetic Modifications and Autoantibody Production

In SLE, rheumatoid arthritis, and other autoimmune disorders, the nucleosome serves as a potent autoantigen. The presence of

nucleosome and histone-specific antibodies, as well as antigen-specific B and T lymphocytes has been demonstrated in the peripheral blood of SLE patients. Nucleosomes can be detected in the serum of SLE patients and lupus-prone mice. It has been suggested that aberrant apoptosis and impaired clearance of apoptotic material or a combination of both contribute to increased nucleosome release into the blood stream. Interestingly, autoantibodies from SLE patients show significantly enhanced activity against acetylated histones from both apoptotic cells and cells treated with HDAC inhibitors which subsequently undergo apoptosis (Table 1). Thus, it appears possible that apoptosis-induced chromatin modifications, such as histone acetylation, are an early event in the loss of self-tolerance, contributing to the development of autoimmune phenotypes (Hedrich and Tsokos 2011). At this point, it can only be speculated whether apoptosis-induced histone motifs may be a suitable substrate for future tolerizing strategies in SLE.

Mechanisms of Epigenetic Dysregulation

The underlying mechanisms leading to disturbed CpG-DNA methylation patterns and histone modifications in autoimmune diseases remain largely unknown. The addition of mitogen-activation protein kinase (MAPK) inhibitors to cultured T and B lymphocytes results in down-regulation of DNMT activity and an increased expression of methylation-sensitive genes, mimicking the pro-inflammatory phenotype of autoreactive lymphocytes. Reduced protein kinase C activity in mice and humans results in reduced MAPK and subsequently reduced DNMT1 activation, subsequently resulting in B lymphocyte expansion and the induction of pro-inflammatory cytokines (Hedrich and Tsokos 2011). Several molecules are involved in CpG-DNA demethylation of T lymphocytes from SLE patients. DNA damage inducible protein alpha (GADD45a), which is over-expressed in T lymphocytes from SLE patients, activation-inducible deaminase (AID), and the methyl-CpG binding domain 4 (MBD-4) related G:T glycosylase have been documented to contribute to CpG-DNA demethylation in B and T lymphocytes from SLE patients, thus contributing to increased gene expression and immune dysregulation (Brooks et al. 2009; Meda et al. 2011; Hedrich and Tsokos 2011; Renaudineau and Youinou 2011). Interestingly, studies investigating DNMT expression in SLE patients have produced conflicting results. This may be the result of variability in disease activity, discrepancies between DNMT mRNA and protein expression, and varying biological activity of DNMTs in the context of different genes, cells, tissues, and diseases (Hedrich and Tsokos 2011; Renaudineau and Youinou 2011) (Table 1).

The knowledge about mechanisms that contribute to pathological histone modifications in autoimmune disease is even more limited. It has been reported that the transcription factor c-AMP response element mediator (CREM)α is increased in T lymphocytes from SLE patients. CREMα is capable of recruiting HDAC1 and DNMT3a to the *IL2* gene, thus mediating epigenetic remodeling and stable silencing of the *IL2* gene. The over-expression of the histone deacetylase Sirtuin-1 in concert with reduced expression of E1A-binding protein p300, P300/CBP-associated factor, and HDAC7 in T lymphocytes from lupus-prone MRL/lpr mice suggest an involvement of these factors in the disruption of the epigenome in autoimmune disorders (Brooks et al. 2009; Meda et al. 2011; Hedrich and Tsokos 2011).

Epigenetic Patterns as Future Therapeutic Target

Given the central involvement of cell- and tissue-specific epigenetic marks in gene regulation and immune homeostasis, a reversion of pathological epigenetic patterns appears to be a promising target for future treatment. Experimental evidence

documenting the efficiency of HDAC inhibitors is growing (Hedrich and Tsokos 2011). However, HDAC inhibitors and other "epigenetic drugs" (including DNMT inhibitors, etc.) mediate genome-wide changes of histone acetylation and CpG-DNA methylation which may also result in dysregulation of previously unaffected genes. Target-directed epigenetic modifiers may reverse epigenetic changes of single genes or genomic regions. Such "intelligent" drugs may be used as future treatment of autoimmune disease and cancer; however, they remain to be discovered.

Conclusions

A constantly growing body of literature supports the central involvement of epigenetic mechanisms in immune programming and its alterations in the development of autoimmune disorders. Attenuated histone acetylation, CpG-DNA methylation, and miRNA expression, resulting in a disruption of immune homeostasis, have been linked to a number of autoimmune diseases, including SLE, Sjögren's syndrome, rheumatoid arthritis, multiple sclerosis, type 1 diabetes, and others.

However, current knowledge is limited and studies are warranted for a better understanding of epigenetic patterns and their contribution to autoimmunity. Epigenetic marks have a potential value as predictive, diagnostic, and prognostic biomarkers for multiple diseases. It is tempting to discuss epigenetic patterns as potential targets for future therapy. Still, currently available "epigenetic treatments," secondary to their global un-targeted mode of action, harbor the risk of disrupting epigenetic marks in unrelated regions, resulting in secondary pathology.

Cross-References

- ▶ CD40
- ▶ Environment and Autoimmunity
- ▶ Interleukin-6
- ▶ Micro-RNA in Autoimmunity
- ▶ Novel Targets in Systemic Lupus Erythematosus
- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Sjögren's Syndrome
- ▶ Skin in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Animal Models
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Systemic Lupus Erythematosus, Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Brenner C, Fuks F. A methylation rendezvous: reader meets writers. Dev Cell. 2007;12(6):843–4.

Brooks WH, Le Dantec C, Pers JO, Youinou P, Renaudineau Y. Epigenetics and autoimmunity. J Autoimmun. 2010;34(3):J207–19.

Chuang JC, Jones PA. Epigenetics and microRNAs. Pediatr Res. 2007;61(5 Pt 2):24R–9.

Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suñer D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci USA. 2005;102(30):10604–9.

Grolleau-Julius A, Ray D, Yung RL. The role of epigenetics in aging and autoimmunity. Clin Rev Allergy Immunol. 2010;39(1):42–50.

Hedrich CM, Tsokos GC. Epigenetic mechanisms in systemic lupus erythematosus and other autoimmune diseases. Trends Mol Med. 2011;17(12):714–24.

Hedrich CM, Rauen T, Tsokos GC. cAMP-responsive element modulator (CREM)α protein signaling mediates epigenetic remodeling of the human interleukin-2 gene: implications in systemic lupus erythematosus. J Biol Chem. 2011;286(50):43429–36. doi: 10.1074/jbc.M111.299339. Epub 2011 Oct 5.

Hofmann SR, Schwarz T, Möller JC, Morbach H, Schnabel A, Rösen-Wolff A, Girschick HJ, Hedrich CM. Chronic non-bacterial osteomyelitis is associated with impaired Sp1 signaling, reduced IL10 promoter phosphorylation, and reduced myeloid IL-10 expression. Clin Immunol. 2011;141(3):317–27.

Hofmann SR, Möller J, Rauen T, Paul D, Gahr M, Rösen-Wolff Z, Brenner S, Hedrich CM. Dynamic CpG-DNA methylation of Il10 and Il19 in CD4+ T lymphocytes and macrophages: effects on tissue-specific gene expression. Klin Padiatr. 2012;224(2):53–60. doi: 10.1055/s-0031-1291359. Epub 2011 Dec 20.

Meda F, Folci M, Baccarelli A, Selmi C. The epigenetics of autoimmunity. Cell Mol Immunol. 2011;8(3): 226–36.

Neidhart M, Rethage J, Kuchen S, Künzler P, Crowl RM, Billingham ME, Gay RE, Gay S. Retrotransposable L1 elements expressed in rheumatoid arthritis synovial tissue: association with genomic DNA hypomethylation and influence on gene expression. Arthritis Rheum. 2000;43(12):2634–47.

Nile CJ, Read RC, Akil M, Duff GW, Wilson AG. Methylation status of a single CpG site in the IL6 promoter is related to IL6 messenger RNA levels and rheumatoid arthritis. Arthritis Rheum. 2008;58(9):2686–93.

Rauen T, Hedrich CM, Juang YT, Tenbrock K, Tsokos GC. cAMP-responsive element modulator (CREM)α protein induces interleukin 17A expression and mediates epigenetic alterations at the interleukin-17A gene locus in patients with systemic lupus erythematosus. J Biol Chem. 2011;286(50):43437–46.

Renaudineau Y, Youinou P. Epigenetics and autoimmunity, with special emphasis on methylation. Keio J Med. 2011;60(1):10–6.

Schoenborn JR, Dorschner MO, Sekimata M, Santer DM, Shnyreva M, Fitzpatrick DR, Stamatoyannopoulos JA, Wilson CB. Comprehensive epigenetic profiling identifies multiple distal regulatory elements directing transcription of the gene encoding interferon-gamma. Nat Immunol. 2007;8(7):732–42.

Stanczyk J, Pedrioli DM, Brentano F, Sanchez-Pernaute O, Kolling C, Gay RE, Detmar M, Gay S, Kyburz D. Altered expression of microRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis Rheum. 2008;58(4): 1001–9.

Sullivan KE, Reddy AB, Dietzmann K, Suriano AR, Kocieda VP, Stewart M, Bhatia M. Epigenetic regulation of tumor necrosis factor alpha. Mol Cell Biol. 2007;27(14):5147–60.

Thai TH, Christiansen PA, Tsokos GC. Is there a link between dysregulated miRNA expression and disease? Discov Med. 2010;10(52):184–94.

Wilson CB, Makar KW, Shnyreva M, Fitzpatrick DR. DNA methylation and the expanding epigenetics of T cell lineage commitment. Semin Immunol. 2005;17(2):105–19.

Zhao M, Wu X, Zhang Q, Luo S, Liang G, Su Y, Tan Y, Lu Q. RFX1 regulates CD70 and CD11a expression in lupus T cells by recruiting the histone methyltransferase SUV39H1. Arthritis Res Ther. 2010;12(6):R227.

Erythema Nodosum

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Synonyms

Chronic erythema nodosum; Erythema contusiformis; Erythema nodosum (EN); Erythema nodosum migrans (EDM); Subacute migratory panniculitis of Vilanova and Piñol

Definition

Erythema nodosum (EN) is a septal panniculitis, which indicates that the inflammation in this disease occurs primarily along the fibrous septa which separate lobules of subcutaneous fat. Generally, the lesions of EN are located symmetrically over the anterior lower extremities. EN may be idiopathic or may be related to an infection, medications, sarcoidosis, inflammatory bowel disease, or other causes (Patterson 2008).

Introduction

EN, the most common type of panniculitis, was first described by Robert Willan during the eighteenth century (Patterson 2008 and Gilchrist and Patterson 2010). It is an inflammation of the fat most commonly seen symmetrically and bilaterally located over the lower legs.

Epidemiology

EN occurs in about 1–5 in every 100,000 individuals. The disease most often affects young

women; the male to female ratio is 1:6 (Schwartz and Nervi 2007). Frequently, it occurs between the second and fourth decades (Gilchrist and Patterson 2010). The highest incidence of the disease occurs between the ages of 20 and 30. The most common causes of the disease vary based on geographical location (Patterson 2008).

Pathophysiology/Immunology

EN is believed to be a type IV delayed hypersensitivity response to various antigens (Gilchrist and Patterson 2010). EN is a cutaneous response to a variety of inciting agents. It is believed that it results from the deposition of immune complexes in and near the septa located in subcutaneous fat. In some studies, patients with EN have been found to have complement activation and circulating immunocomplexes, whereas in other studies, patients were not found to have these immunocomplexes (Requena and SanchezYus 2008).

Presentation

This panniculitis presents with circular or oval tender cutaneous nodules. They are usually located symmetrically on the anterior lower legs. Lesions may also be distributed along the thighs or arms. They generally range in size from 1 to larger than 6 cm, and they usually resolve anywhere between 1 and 6 weeks after initial onset (Mana and Marcoval 2007). Episodes may last longer and recurrences are possible (Requena and SanchezYus 2008).

Lesions are usually firm, elevated, red, warm, shiny, and smooth during the initial presentation. As the lesions evolve, they flatten and leave a bruise-like appearance (erythema contusiformis). Of note, the lesions of EN do not scar or cause residual atrophy. The lesions tend to occur in crops and then slowly resolve (James et al. 2005). One to three weeks prior to the onset of cutaneous lesions, a prodrome may occur. During this period, individuals may present with muscle and joint aches, fevers, weight loss, fatigue, and cough (Schwartz and Nervi 2007). Abdominal symptoms including pain, vomiting, and diarrhea may be present. Patients may develop episcleral lesions or conjunctivitis. Less commonly, lymphadenopathy, hepatomegaly, splenomegaly, or pleuritis may occur (Requena and SanchezYus 2008). Depending on the underlying etiology, other systemic symptoms may be present as well. Patients may also develop leukocytosis and increased erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (Schwartz and Nervi 2007).

There is a chronic variant of erythema nodosum which may be referred to as chronic EN, EN migrans, or subacute migratory panniculitis of Vilanova and Piñol. Chronic EN is less frequently seen than the acute form and is characterized by lesions that last months to years. This form may appear as a single lesion that improves but travels centrifugally. Ultimately, annular subcutaneous plaques are seen. In chronic EN, lesions affect the body in an asymmetric or unilateral manner and are not as painful as lesions in acute EN. Compared to acute EN, chronic EN is seen in older women and, with the exception of arthralgias, is generally not associated with systemic symptoms or systemic disease (James 2005).

In children and young adults, another variant of EN may occur. This form usually presents unilaterally on the palms or soles and usually occurs following physical activity (Requena and SanchezYus 2008).

Causes

EN can be idiopathic or occur secondary to a number of different etiologic factors. Broadly, these etiologic factors fall under the categories of infectious, medication-related, malignancy-related, and other miscellaneous entities (Requena and SanchezYus 2008) (Table 1).

Erythema Nodosum, Table 1 Major causes of erythema nodosum

Cause	Notes
Idiopathic	Often, a cause for EN is not found (Patterson 2008)
Infectious	**Bacteria** • In kids, streptococcal (Lancefield Group A, Streptococcus pyogenes) pharyngitis is the most common cause of EN (Gilchrist and Patterson 2010) • Bacterial gastrointestinal infections implicated in EN include Yersinia, Salmonella, Shigella, Campylobacter (James et al. 2005 and Gilchrist and Patterson 2010) **Fungal** • EN may be seen in coccidioidomycosis. When associated with EN, coccidioidomycosis is generally not disseminated (Patterson 2008) • Other less common causes include histoplasmosis and blastomycosis (Patterson 2008) **Mycobacteria** • Tuberculosis (TB) should be suspected in regions with high prevalence of the disease. However, TB is an uncommon cause of EN in areas where TB is rare (Mert et al. 2004)
Malignancy	This is usually a rarer cause, but EN can be seen in Hodgkin disease and acute myelogenous leukemia (Patterson 2008)
Sarcoidosis	EN almost always occurs during the acute phase of sarcoidosis associated with bilateral hilar lymphadenopathy, otherwise known as Löfgren's syndrome. However, occasionally, EN may occur in chronic sarcoidosis (Mana and Marcoval 2012) **Löfgren's syndrome** • This is an acute presentation of sarcoidosis and consists of EN, hilar lymphadenopathy, fevers, cough, and arthralgias (James et al. 2005). In addition, anterior uveitis, pulmonary involvement, and periarticular ankle inflammation may be seen. It is seen most often in young women from the Nordic region (Mana et al. 1999). The syndrome has a good prognosis; it is often self-limited and responsive to treatment (James et al. 2005)
Medications	Bromides Sulfonamides Oral contraceptive pills(OCP) • Although with a decrease in the hormonal content of more recent OCPs, they are becoming a rarer cause (Requena and SanchezYus 2008) Penicillin(Psychos et al. 2000)
Inflammatory bowel disease	Crohn's disease Ulcerative colitis
Behcet's disease	Behcet's disease often starts with oral ulceration and progresses to involve other mucocutaneous findings, including EN. This disease also has other systemic involvement including eyes, vessels, and digestive and nervous systems (Kaneko et al. 2011)
Pregnancy	Estrogens may be associated with the development of EN. It is often seen in the first or second trimesters of pregnancy (Mert et al. 2004)
Others	Connective tissue diseases Other infectious diseases

Diagnosis

History and clinical presentation may provide enough clues to make a diagnosis of EN. However, a skin biopsy may be diagnostic. A deep incisional biopsy or a double-punch biopsy may be necessary to capture the pathology (Gilchrist and Patterson 2010).

The histology of EN reveals a septal panniculitis. Distinguishing EN as a septal panniculitis is important as it greatly narrows the differential diagnosis and separates EN from lobular panniculitides which can sometimes have similar clinical presentations. In EN, different cells may be present, including histiocytes, lymphocytes, neutrophils, and sometimes eosinophils (Rapini 2005). The subcutaneous fat septa are thickened and are the primary location of the inflammatory infiltrate. However, the inflammatory cells may extend into the periseptal areas within the fat lobules. Early-stage EN lesions are characterized by neutrophils, edema, and hemorrhage. In long-standing lesions, multinucleated

giant cells, lymphocytes, histiocytes, granulation tissue, and fibrosis are seen (Requena and SanchezYus 2008). Immunoglobulin deposition in the vessel walls within septa has been demonstrated through direct immunofluorescence studies (Requena and SanchezYus 2008).

Preliminary investigations should consist of complete blood count including differential erythrocyte sedimentation rate, antistreptolysin O titer, urinalysis, throat culture, tuberculosis testing, and chest radiograph (Requena and SanchezYus 2008). Stool examinations and blood cultures may be obtained depending on patient presentation and history (Schwartz and Nervi 2007).

Differential Diagnosis

The differential diagnosis of EN includes other panniculitides such as lupus profundus, cold panniculitis, poststeroid panniculitis, or sclerosing panniculitis. In addition, cytophagic histiocytic panniculitis and leukemic fat infiltrates are among neoplastic entities that may mimic EN. Deep fungal, bacterial, and mycobacterial infections may mimic the presentation of EN. Other diseases that should be considered include alpha 1-antitrypsin deficiency, nodular fat necrosis, necrobiosis lipoidica, necrobiotic xanthogranuloma, scleroderma, subcutaneous granuloma, and other panniculitides (Schwartz and Nervi 2007). EN may be differentiated from these entities through clinical presentation, histological examination, and direct immunofluorescence studies.

Treatment and Management

The first approach toward treating EN is to eliminate the inciting trigger. It is important to look through the patient's history and remove possible offending medications. It is imperative to treat possible infections or malignancies. Generally, EN is self-limited so addressing symptoms is the next step. It may take up to 2 months before new EN lesions stop developing (Gilchrist and Patterson 2010).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are considered first-line treatment. Therapy is started with indomethacin. Ibuprofen or naproxen may be used in patients who do not respond to indomethacin. Patients are advised to get adequate rest, elevate the legs for half an hour twice a day, and use compression stockings (Gilchrist and Patterson 2010).

Potassium iodide (KI), dispensed as saturated solution of KI (SSKI), is used to treat erythema nodosum. The mechanism is not completely understood; however, it is thought that SSKI may work by inhibiting neutrophil chemotaxis. Adults generally are started at a dose of 300 mg three times daily and titrated up to 500 mg three times daily if needed. Patients may note improvement around 14 days. Side effects include nausea, distortion of taste, hypersalivation, and irritation of the GI tract. Decreasing the dose can reduce these side effects. Patients are often encouraged to take the medication with orange juice (Gilchrist and Patterson 2010). This medication is contraindicated in pregnancy as it may cause the growth of a goiter in the fetus. Additionally, significant hypothyroidism may occur as a result of taking this medication (Requena and SanchezYus 2008).

Colchicine is another option in treating EN. It is particularly helpful in patients with Behcet's disease who develop lesions of EN. Females tend to respond to the medication more than men. Colchicine is anti-inflammatory and arrests microtubule polymerization, thereby impairing neutrophil chemotaxis. Gastrointestinal side effects are common and include diarrhea and abdominal pain. Starting colchicine at a low dose, such as 0.6 mg once daily, and increasing to twice daily as tolerated helps with these side effects. Bone marrow suppression with pancytopenia may occur with overdose (Gilchrist and Patterson 2010).

After malignancy and underlying infection have been ruled out, systemic steroid treatment may provide therapeutic benefit. In EN, systemic steroids are dosed at 1 mg per kg (Schwartz and Nervi 2007).

Local treatment of EN includes intralesional injections of triamcinolone acetonide. The

medication is injected into the center of lesions at a concentration of 5 mg/ml (Requena and SanchezYus 2008).

Conclusion

EN can occur from a number of causes. Most often it occurs idiopathically. However, if an underlying cause does exist, finding and treating the triggering disease is the first step in clearing the eruption.

Cross-References

► Sarcoidosis

References

Gilchrist H, Patterson JW. Erythema nodosum and erythema induratum (nodular vasculitis): diagnosis and management. Dermatol Ther. 2010;23:320–7.

James W, Berger T, Elston D. Diseases of subcutaneous fat. In: Odom RB, James WD, Berger T, editors. Andrews' diseases of the skin: clinical dermatology. 10th ed. Philadelphia: Saunders; 2005. p. 487–97.

Kaneko F, Togashi A, Saito S, Sakuma H, Oyama N, Nakamura K, Yokota K, Oguma K. Behcet's disease (Adamantiades-Behcet's disease) Clin Dev Immun. 2011; 2011:681956.

Mana J, Gomez-Vaquero C, Montero A, Salazar A, Marcoval J, Valverde J, Manrea F, Pujol R. Lofgren's syndrome revisited: a study of 186 patients. An J Med. 1999;107:240–5.

Mana J, Marcoval J. Erythema Nodosum. Clin Dermatol. 2007;25:288–94.

Mana J, Marcoval J. Skin manifestations of sarcoidosis. Presse Med. 2012;41(6 Pt 2):e355–74.

Mert A, Ozaras R, Tabak F, Pekmezci S, Demirkesen C, Ozturk R. Erythema nodosum: an experience of 10 years. Scand J Infect Dis. 2004;36:424–7.

Patterson JW. Panniculitis. In: Bolognia JL, Jorizzo JL, Rapini RP, editors. Dermatology. London: Elsevier; 2008. p. 1515–35.

Psychos DN, Voulgari PV, Skopouli FN, Drosos AA, Moutsopoulos HM. Erythema nodosum: the underlying conditions. Clin Rheumatol. 2000;19:212–6.

Rapini RP. Eczematous and papulosquamous diseases. In: Rapini RP, editor. Practical dermatopathology. Philadelphia: Elsevier; 2005. p. 45–59.

Requena L, SanchezYus E. Erythema nodosum. Dermatol Clin. 2008;26:425–38.

Schwartz R, Nervi S. Erythema nodosum: a sign of systemic disease. Am Fam Physician. 2007;75: 695–700.

F

Fas/Fas Ligand

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Synonyms

Fas: CD95, Apo-1; Fasl: CD95 ligand

Definition

Fas and Fas ligand (Fasl) are transmembrane proteins that belong to the TNF receptor family and TNF family of proteins, respectively.

Fas (CD95) is expressed on many types of cells, yielding them susceptible of killing by cells expressing Fasl.

Fasl (CD95l) is expressed mainly on activated T cells. Binding of Fasl triggers apoptosis (**programmed cell death**) in the Fas-expressing cells.

Fas/Fasl: Mediators of Apoptosis

Fas is a transmembrane receptor ubiquitously expressed, in particular, in brain, heart, kidney, liver, pancreas, thymus, and lymphoid tissues. Fasl is a protein expressed mainly on membranes of activated T lymphocytes. The interaction between Fasl and the Fas receptor, triggers apoptotic signals in the cell expressing Fas (Elgert 2009a; Hotchkiss et al. 2009). The ligation of Fas by Fasl causes the activation of target cell enzymes to degrade target cell nuclear DNA with concomitant fragmentation of the target cell nucleus, leading to programmed cell death. When Fasl binds Fas, Fas trimerizes and activates its "death" domain that interacts with the "death" domains of several cytosolic proteins including FADD (Fas-associated death domain). Activation of FADD triggers the activation of a series of cysteine proteases known as caspases, resulting in apoptosis of the cell with the consequent morphological changes (reduction of cell volume, plasma membrane blebbing, condensation of chromatin, and fragmentation of DNA) (Elgert 2009a; Hotchkiss et al. 2009; Guicciardi and Gores 2009) (Fig. 1). Phosphatidylserine, normally found on the cytosolic surface of plasma membranes, is redistributed during the process of apoptosis to the extracellular surface. Phagocytic cells recognize this aberrant placement and remove the dying cells without the induction of inflammation. Cell removal is followed by a reset of the activated T lymphocytes to initiate another Fas/Fasl interaction (Elgert 2009a; Hotchkiss et al. 2009).

Fas-mediated apoptosis plays a critical role in the removal of mature autoreactive B and T lymphocytes as well as in the elimination of infected or malignant cells (Elgert 2009b, c; Crow 2003; Kay 2003).

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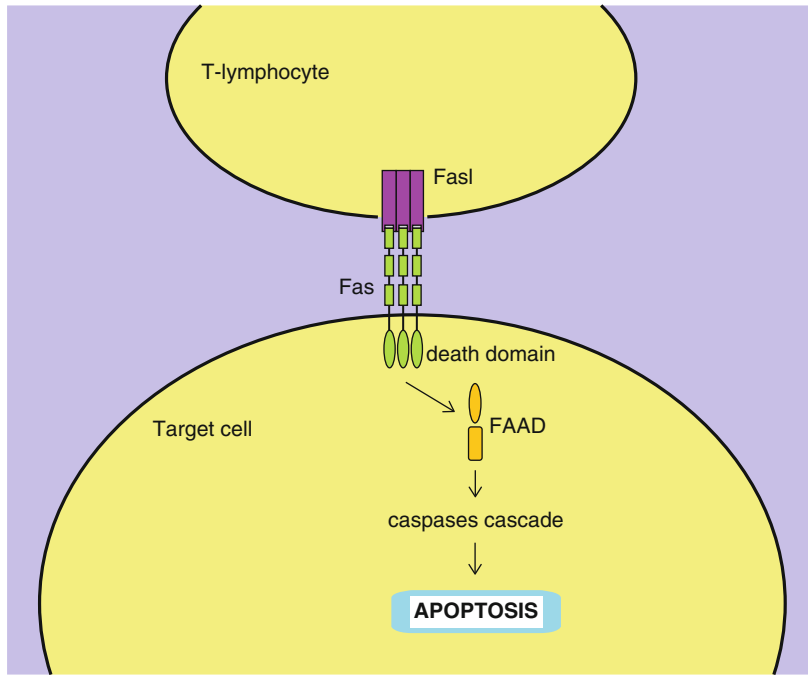

Fas/Fas Ligand, Fig. 1 *Fas/Fasl apoptotic pathway*. The interaction between Fasl (expressed on T-activated lymphocyte) and the Fas receptor, expressed on target cell, triggers apoptotic signals in the cell expressing Fas. After binding of Fas by Fasl, Fas trimerizes and activates its "death" domain that interacts with the Fas-associated death domain protein (*FADD*). Therefore, activation of FADD triggers the activation of the caspases cascade, resulting in programmed cell death

Fas/Fasl: Key Role in Autoimmunity

The biological importance of Fas/Fasl interaction in the development of autoimmune diseases was established in naturally occurring mutations of either Fas or Fasl proteins in mice. The genetic lesions lead to massive T lymphocyte proliferation in lymph nodes and spleen. The mouse strain with a lesion in the gene encoding Fasl is called *gld* (general lymphoproliferative disorder). These mice do not exhibit Fas-/Fasl-induced apoptosis and therefore develop autoimmune disease early in life (Coico and Sunshine 2009). A human disease which mimics the murine model is the autoimmune lymphoproliferative syndrome (ALPS), caused by a mutation in the genes encoding Fas and Fasl. This syndrome is characterized by lymphoproliferation (lymphadenopathy, hepatosplenomegaly), several autoimmune manifestations (mainly autoimmune cytopenias), and an increased propensity to malignancy (lymphomas) (Worth et al. 2006; Teachey et al. 2010). ALPS is the first human disease which etiology has been attributed to a primary defect in apoptosis, linking lymphocyte homeostasis and the autoimmune response (Turbyville and Rao 2010; Su and Anderson 2009). Considering that abnormalities in apoptosis can increase susceptibility to autoimmunity, defects in apoptosis and the clearance of apoptotic cells have been linked to several autoimmune diseases (Elgert 2009b; Crow 2003; Viorritto et al. 2007).

Normally, in order to eliminate autoreactive T cells (mature T lymphocytes that recognize self-antigens), interaction between autoreactive T cell Fas and activated T lymphocytes Fasl induces apoptosis of the autoreactive T cells (Strasser and Pellegrini 2004). This negative regulation of T cells contributes to the elimination of T lymphocyte-activated autoreactive B cells, in the absence of presentation of the autoantigen by the autoreactive T cell (Elgert 2009b; Crow 2003; Rathmell et al. 1996). By virtue of elimination of these cells, the immune system remains safe and effective. Thus, normal Fas/Fasl function results in normal lymphocyte homeostasis and controlled autoreactivity. An alteration in Fas/Fasl structure results in impaired immune

tolerance and in uncontrolled lymphoproliferation (Turbyville and Rao 2010; Su and Anderson 2009; Hughes et al. 2008). Inadequate removal of self-reactive T lymphocytes permits the production of pathogenic autoantibodies that characterize autoimmune diseases (Crow 2003; Strasser and Pellegrini 2004; Rathmell et al. 1996).

Fas/FasL: Role in Tumor Surveillance

A critical function of the immune system is to control of malignant cell proliferation. In fact, patients with immunodeficiencies and/or autoimmune diseases are at increased risk for development of tumors. More than 50 % of neoplasms exhibit defects in apoptosis (Elgert 2009c).

The Fas/FasL proteins may be considered oncogenes, as malignancies can develop with Fas/FasL mutations. These mutations increase the capability of malignant cells to avoid immune surveillance (Elgert 2009c; Peter et al. 2005). Patients with ALPS (main sample of a disease associated with an apoptotic defect) are susceptible to malignant transformation of their lymphocytes due to high rates of cell division in the absence of normal Fas-/FasL-mediated homeostasis (Worth et al. 2006).

The evolution of tumor cells to a more malignant phenotype requires the acquisition of genetic changes that subvert apoptosis pathways and promote cancer cell survival (Elgert 2009c). It has been assumed that Fas and FasL work as tumor suppressors, since mutations that downregulate normal function of Fas have been proposed as a mechanism by which tumor cells avoid apoptosis or destruction by the immune system (Elgert 2009c). However, a single point mutation of the Fas gene can change Fas/FasL interaction from tumor suppression to tumor promotion by induction of prosurvival genes through non-apoptotic pathways. This dual role of Fas/FasL is found in advanced cancer in humans, resulting in apoptosis resistance and activation of tumorigenic pathways (Peter et al. 2005).

Fas/FasL: Biomarkers of Inflammation

Inflammatory biomarkers might help to identify specific inflammatory disturbances. Therefore, targeting specific biomarkers of inflammation might represent new therapeutic approaches (Schütz et al. 2010). Both Fas and FasL proteins exert a wide range of proinflammatory functions by inducing secretion of cytokines and chemokines (Kay 2003). Beyond activating apoptosis, the activation of the Fas "death domain" can initiate multiple non-apoptotic signaling pathways, including inflammatory responses (Hotchkiss et al. 2009; Guicciardi and Gores 2009). Furthermore, Fas/FasL increases cell removal from areas of chronic inflammation (Guicciardi and Gores 2009).

An example of the importance of Fas/FasL in inflammation is the development and progression of heart failure. Fas is expressed in cardiac myocytes, and serum concentrations of a soluble form of Fas are elevated in patients with heart failure. Furthermore, high levels of soluble Fas are associated with more severe disease, and inhibition of Fas in animal models reduces post infarction syndrome and improves survival (Braunwald 2008). Measurement of Fas/FasL soluble forms appears to be useful in risk stratification of patients with heart failure and in the screening of asymptomatic patients to identify heart failure risk. Pharmacological reduction of Fas levels may represent a new direction for treatment or prevention of heart failure (Braunwald 2008).

Cross-References

▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Braunwald E. Biomarkers in heart failure. N Engl J Med. 2008;358:2148–59.

Coico R, Sunshine G. Tolerance and autoimmunity. In: Immunology: a short course. New York: Wiley; 2009. p. 183–204.

Crow MK. Cellular immunology. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Wisman MH, editors. Rheumatology. Edinburgh: Mosby; 2003. p. 1347–58.
Elgert KD. Cellular interactions: development of effector functions and their regulation. In: Immunology: understanding the immune system. New York: Wiley; 2009. p. 321–68.
Elgert KD. Immunologic tolerance and autoimmunity. In: Immunology: understanding the immune system. New York: Wiley; 2009a. p. 431–60.
Elgert KD. Tumor immunology. In: Immunology: understanding the immune system. New York: Wiley; 2009b. p. 489–528.
Guicciardi ME, Gores GJ. Life and death by death receptors. FASEB J. 2009;23:1625–37.
Hotchkiss RS, Strasser A, et al. Cell death. N Engl J Med. 2009;361:1570–83.
Hughes PD, Belz GT, et al. Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. Immunity. 2008;28:197–205.
Kay RA. The principles of adaptative immunity. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Wisman MH, editors. Rheumatology. Edinburgh: Mosby; 2003. p. 113–26.
Peter ME, Legembre P, et al. Does CD95 have tumor promoting activities? Biochim Biophys Acta. 2005;1755:25–36.
Rathmell JC, Townsend SE, et al. Expansion or elimination of B cells in vivo: dual roles for CD40- and Fas (CD95)-ligands modulated by the B cell antigen receptor. Cell. 1996;87:319–29.
Schütz C, Oelke M, et al. Killer artificial antigen-presenting cells: the synthetic embodiment of a 'guided missile'. Immunotherapy. 2010;2:539–50.
Strasser A, Pellegrini M. T-lymphocyte death during shutdown of an immune response. Trends Immunol. 2004;25:610–5.
Su MA, Anderson MS. Monogenic autoimmune diseases: insights into self-tolerance. Pediatr Res. 2009;65:20R–5.
Teachey DT, Seif AE, et al. Advances in the management and understanding of autoimmune lymphoproliferative syndrome (ALPS). Br J Haematol. 2010;148:205–16.
Turbyville JC, Rao VK. The autoimmune lymphoproliferative syndrome: A rare disorder providing clues about normal tolerance. Autoimmun Rev. 2010;9:488–93.
Viorritto IC, Nikolov NP, et al. Autoimmunity versus tolerance: can dying cells tip the balance? Clin Immunol. 2007;122:125–34.
Worth A, Thrasher AJ, et al. Autoimmune lymphoproliferative syndrome: molecular basis of disease and clinical phenotype. Br J Haematol. 2006;133:124–40.

FcγRIIB

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Synonyms

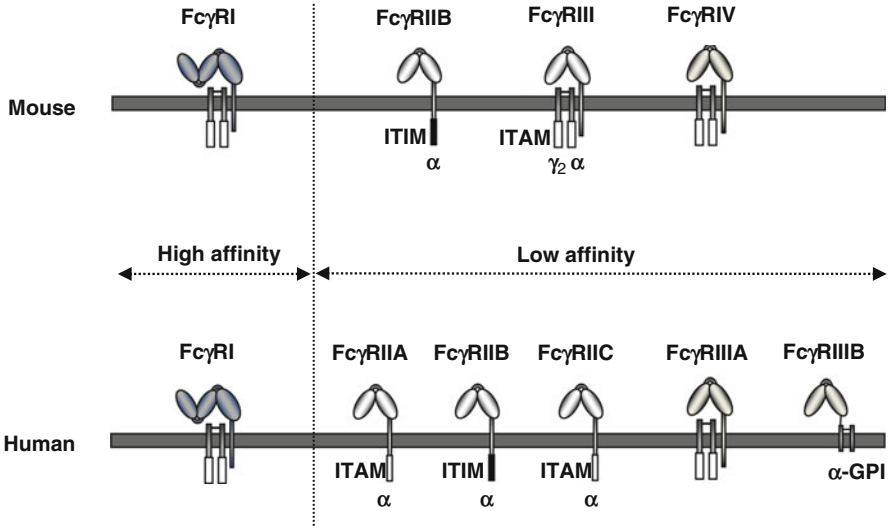
CD32B; Fc fragment of IgG low-affinity IIb receptor

Definition

FcγRIIB is a low-affinity receptor ($K_a = 10^7\ M^{-1}$) for the constant region of immunoglobulin G (IgG) and belongs to the family of Fcγ-receptors (FcγR).

Structure and Expression

The 40 kDa glycoprotein consists of two immunoglobulin-like extracellular domains, a transmembrane region and a cytoplasmic tail containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) (Fig. 1). In humans and mice, two isoforms of FcγRIIB exist as a result of alternative splicing, designated FcγRIIB1 and FcγRIIB2. An insertion of 19 amino acids in humans and 47 amino acids in mice in the FcγRIIB1 isoform interrupts an amino acid motif in the cytoplasmic tail essential for endocytosis. Whereas FcγRIIB2 expression is restricted to myeloid cells, FcγRIIB1 is the main form expressed on B cells. On the majority of immune cells, including macrophages, mast cells, neutrophils, dendritic cells (DCs), and monocytes, FcγRIIB is co-expressed with activating FcγRs (FcγRI, FcγRIII, and FcγRIV in the



FcγRIIB, Fig. 1 The family of Fcγ-receptors in mouse and humans. Depicted are the members of the mouse and human FcγR family. The individual family members can be distinguished by the signals they transduce and by the affinity for IgG. FcγRIIB is a low-affinity receptor for IgG, which is characterized by an ITIM motif in its cytoplasmic domain

mouse and FcγRIA, FcγRIIA, and FcγRIIIA in humans) and thereby sets a threshold for cell activation. In contrast, FcγRIIB is the only FcγR expressed on B cells where it regulates positive signals transmitted by the B cell receptor (Ravetch and Nimmerjahn 2008).

The expression level of FcγRIIB on different B cell developmental stages varies between mice and men. In the mouse, FcγRIIB is expressed starting from the pre-B cell stage (Foy et al. 1992). In comparison to naive B cells, germinal center B cells (GC B cells) show a lower expression of FcγRIIB, whereas in plasma cells a higher expression was observed in mice (Jiang et al. 1999). In most of the human studies using isolated B cells from human blood, memory B cells show a higher expression of FcγRIIB than naive B cells (Isaak et al. 2008; Mackay et al. 2006; Tackenberg et al. 2009). For plasma cells, one study showed a lower expression of FcγRIIB compared to naive B cells (Catalan et al. 2010). On B cells from human spleen and B cells from NOD/Scid/$\gamma c^{-/-}$-deficient mice reconstituted with human CD34+ hematopoietic stem cells, memory B cells and plasmablasts had a higher expression of FcγRIIB than naive B cells, consistent with the data obtained in mice. In contrast to the mouse, however, human germinal center B cells had a higher expression of FcγRIIB than naive B cells, and the analysis of human bone marrow showed that FcγRIIB expression starts at the level of immature B cells (Baerenwaldt et al. 2011). Compared to B cells, monocytes, neutrophils, and DCs have a lower amount of FcγRIIB on the cell surface (Su et al. 2007). However, FcγRIIB expression can be modulated by several cytokines or bacterial components. Incubation of monocyte-derived DCs (moDCs) or plasmacytoid DCs with IL-4 and IL-10 was shown to up-regulate FcγRIIB expression (Su et al. 2007; Wijngaarden et al. 2004). On monocytes, IL-4 enhances FcγRIIB expression, whereas it leads to a decline of FcγRIIB on B cells (Rudge et al. 2002). In contrast to IL-4, IFN-γ treatment of monocytes, moDCs, and B cells leads to a reduced expression of FcγRIIB. TNF-α and LPS provoked

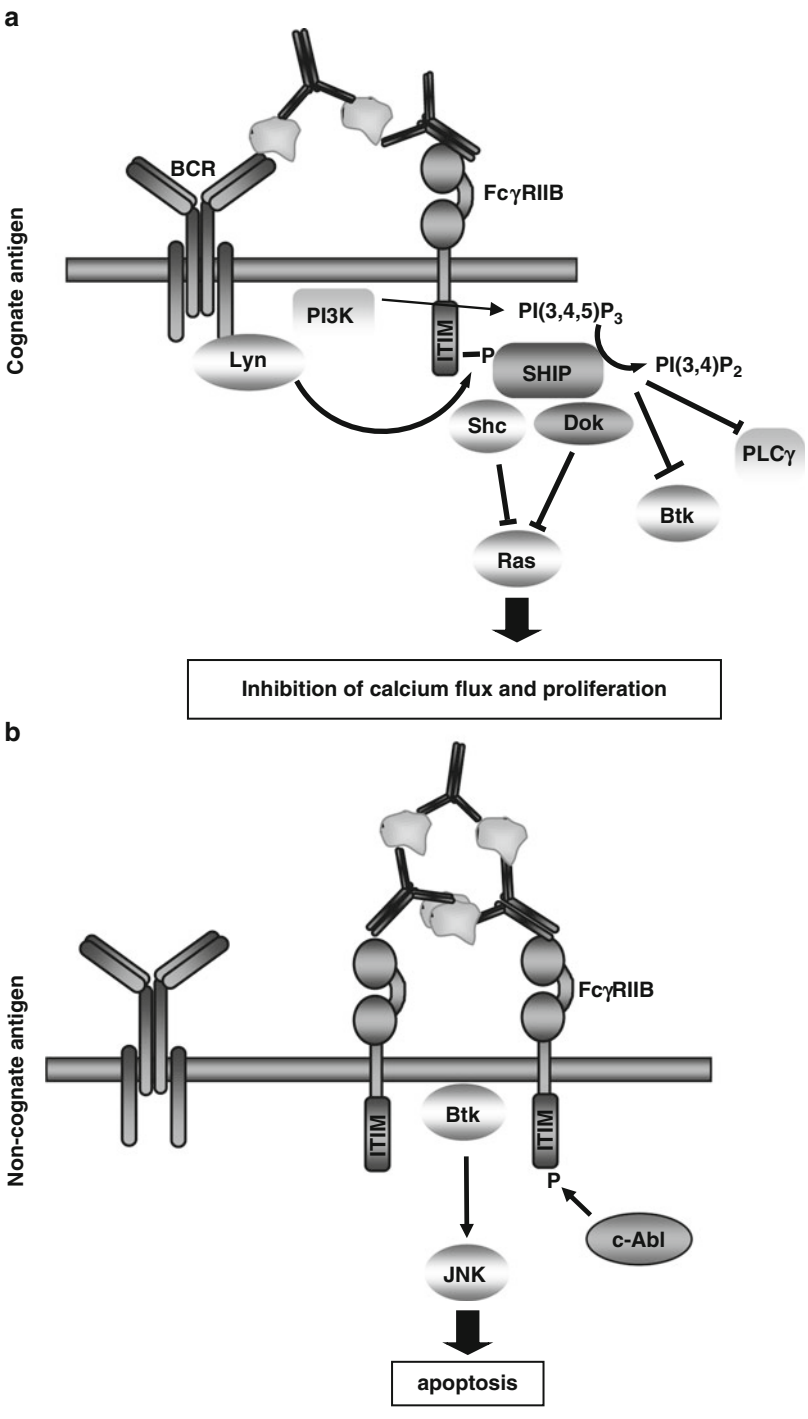
a down-modulation of FcγRIIB on moDCs (Boruchov et al. 2005). Therapeutic agents can also alter the expression of FcγRIIB. Thus, intravenous immunoglobulin (IVIg), which is used for the treatment of several autoimmune diseases, was shown to up-regulate FcγRIIB on monocytes, naive B cells, and memory B cells in humans (Tackenberg et al. 2010).

Signaling of FcγRIIB

There are two major pathways, by which FcγRIIB can transmit signals into the cell, which are either dependent or independent of the ITIM motif in the cytoplasmic tail (Fig. 2). In the ITIM-dependent pathway, immune complex binding to FcγRIIB in combination with activating signals transduced by the B cell receptor or other activating FcγRs leads to the phosphorylation of the ITIM motif by the kinase Lyn, leading to the recruitment of SRC-homology-2 domain-containing inositol polyphosphate-5-phosphatase (SHIP). This results in the dephosphorylation of phosphatidyl-inositol (3,4,5)-trisphosphate (PIP_3) which subsequently inhibits the association of Bruton's tyrosine kinase (Btk) and phospholipase C gamma (PLCγ). The outcome of this signaling pathway is the reduction of calcium influx and a diminished activation signal initiated through activating receptors. The second signaling pathway is independent of the ITIM motif and seems to be restricted to B cells. Cross-linking of FcγRIIB without co-cross-linking of the B cell receptor induces a SHIP-independent but Btk- and c-Abl-dependent signaling pathway ultimately leading to apoptosis (Tarasenko et al. 2007). This pathway is believed to be important in the germinal center reaction to delete low-affinity (and thus potentially autoreactive) B cells and to control plasma cell persistence (Xiang et al. 2007).

Modulation of Immune Cells by FcγRIIB

FcγRIIB modulates immune cells by setting a threshold for cell activation. Only if the activating signal is strong enough to overcome the inhibitory signal induced by FcγRIIB, cells can become activated. On innate immune effector cells, where FcγRIIB is co-expressed with activating FcγRs, antibody-triggered effector functions such as antigen presentation, release of cytokines, or induction of the respiratory burst are controlled by this threshold. On B cells, FcγRIIB is co-cross-linked with the B cell receptor if the antigen is presented in form of IgG immune complexes, resulting in the inhibition of B cell activation and proliferation. Modulation of B cell and innate effector cell activation by FcγRIIB was shown to be an important mechanism to limit and shut down antibody-mediated immune responses. In case of innate effector cells, FcγRIIB prevents the continuous production of inflammatory cytokines induced by cross-linking of activating FcγRs. As a result, the inflammatory response induced by the antigen can be shut down after a successful immune response (Ravetch and Nimmerjahn 2008). With respect to B cells, the threshold set by FcγRIIB is believed to be of major importance especially in the germinal center reaction. Here, B cells activated by their specific antigen undergo somatic hypermutation (SHM) to create BCRs (and later antibodies) of high affinity and narrow specificity. Since this process is driven by chance, it has to be tightly regulated to prevent the generation of autoreactive BCRs and antibodies. In the germinal center antigen is presented by follicular dendritic cells in form of immune complexes. This results in co-ligation of FcγRIIB with the BCR on antigen-specific B cells, establishing a threshold set by positive and negative signaling events. If SHM of the BCR results in a high BCR affinity for the cognate antigen, the B cell gets a strong activation signal that overcomes the negative threshold set by FcγRIIB and is positively selected (Fig. 2a). If SHM results in the loss of antigen recognition, the inhibitory signal of FcγRIIB is the only signal transmitted to the B cell leading to the induction of apoptosis (Fig. 2b). Thus, FcγRIIB-dependent elimination of B cells that lost their antigen specificity may be important to delete potentially autoreactive B cell species (Nimmerjahn and Ravetch 2010).



FcγRIIB, Fig. 2 Signaling pathways induced by FcγRIIB cross-linking. (**a**) Co-cross-linking of FcγRIIB with the B cell receptor (BCR) or activating FcγRs (not depicted) results in a Lyn-dependent phosphorylation of the ITIM motif in the cytoplasmic tail of the inhibitory FcγRIIB. This recruits SHIP to the ITIM domain, leading to the inhibition of downstream activating signaling pathways, such as the release of calcium. (**b**) Isolated triggering of the inhibitory FcγRIIB on B cells was shown to induce apoptosis in an ITIM-independent but Btk- and c-Abl-dependent manner. See text for further details

Role of FcγRIIB in Autoimmunity

Over the last years, several studies using either mouse model systems or human autoimmune patient cohorts suggest that FcγRIIB may be crucially involved in maintaining humoral tolerance. Mouse strains like BXSB, NZB, MRL, or NOD that develop spontaneous autoimmune diseases

were shown to have deletions in the promoter region of FcγRIIB leading to a diminished expression of FcγRIIB on germinal center B cells. Deletion of FcγRIIB in the mouse results in a higher susceptibility to develop spontaneous or induced autoimmunity. Thus, injection of immune complexes into FcγRIIB−/− mice results in development of glomerulonephritis or alveolitis, and immunization with collagen leads to the development of Goodpasture's syndrome and arthritis on usually non-susceptible genetic backgrounds. On the C57Bl/6 background deletion of FcγRIIB results in the spontaneous development of autoimmunity, characterized by the production of anti-DNA antibodies and the development of glomerulonephritis. Cell type-specific overexpression of FcγRIIB on B cells but not on macrophages, was able to restore self-tolerance (Brownlie et al. 2008).

In humans, two polymorphisms in the *fcgr2b* gene were associated with autoimmunity. In the Caucasian population, a promoter polymorphism was associated with systemic lupus erythematosus (SLE). The exchange of guanine to cytosine on position −386 is believed to influence the expression of FcγRIIB, but there are conflicting results if this promoter variant enhances or decreases the expression. In the Asian as well as in the Caucasian population, a polymorphism in the transmembrane region of FcγRIIB was shown to be present at a higher frequency in SLE patients compared to healthy donors. This polymorphism leads to an exchange of the isoleucine residue at position 232 (in the transmembrane domain of FcγRIIB) with a threonine residue, leading to a diminished recruitment of this receptor variant into lipid rafts upon co-cross-linking with activating receptors. Functionally this translates into a decreased inhibitory signal, an increased calcium flux, and a higher degree of cell activation (Tarasenko et al. 2007). In addition, several studies comparing cells of autoimmune patients with healthy donors support a role of FcγRIIB for human B cell tolerance. Thus, B cells from patients with different autoimmune diseases seem to have an altered expression of FcγRIIB. B cells of SLE patients (Mackay et al. 2006), patients with rheumatoid arthritis (Catalan et al. 2010), and chronic inflammatory demyelinating polyneuropathy (CIDP) (Tackenberg et al. 2009) have a reduced expression of FcγRIIB compared to healthy controls. Furthermore, the up-regulation of FcγRIIB from naive to memory B cells which can be observed in healthy individuals was absent on memory B cells of SLE and CIDP patients (Mackay et al. 2006; Tackenberg et al. 2009). Several lines of evidence suggest that FcγRIIB represents a late tolerance checkpoint during B cell development. Thus, an increased number of plasma cells, memory B cells, or GC B cells can be detected in FcγRIIB$^{-/-}$ mice, humanized mice, and in autoimmune patients (Baerenwaldt et al. 2011; Tarasenko et al. 2007). Furthermore, an increased number of autoantibody-producing cells can also be detected in FcγRIIB$^{-/-}$ mice (Fukuyama et al. 2005; Rahman et al. 2007). Additionally, it was shown that autoreactive B cells in the spleen remain at the border of the T cell zone and B cell zone and do not participate in the germinal center reaction in an FcγRIIB-dependent manner (Paul et al. 2007).

Conclusion

FcγRIIB is a low-affinity receptor for the constant region of IgG. Within the family of FcγRs, it is the only inhibitory receptor containing an ITIM motif in its cytoplasmic domain. The co-aggregation of FcγRIIB with ITAM bearing receptors, like activating FcγRs or the BCR, sets a threshold for cell activation and modulates the outcome of the resulting cellular response. In the humoral immune response, FcγRIIB is crucial for controlling the activation and proliferation of antigen-specific B cells and may represent a major checkpoint for humoral tolerance in mice and in humans. In the efferent phase of an antibody-dependent immune response, FcγRIIB modulates innate immune effector cell activation

resulting in a balanced pro-inflammatory response. New approaches influencing FcγRIIB expression on different cell types may help to treat autoimmune diseases and chronic inflammation in the future.

Cross-References

- ▶ Animal Models in Rheumatoid Arthritis
- ▶ Autoantibodies in Rheumatoid Arthritis
- ▶ Autoinflammatory Diseases
- ▶ Novel Targets in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Animal Models
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Baerenwaldt A, Lux A, Danzer H, Spriewald BM, Ullrich E, Heidkamp G, Dudziak D, Nimmerjahn F. Fcgamma receptor IIB (FcgammaRIIB) maintains humoral tolerance in the human immune system in vivo. Proc Natl Acad Sci USA. 2011;108:18772–7.

Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV, Young JW. Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. J Clin Invest. 2005;115:2914–23.

Brownlie RJ, Lawlor KE, Niederer HA, Cutler AJ, Xiang Z, Clatworthy MR, Floto RA, Greaves DR, Lyons PA, Smith KG. Distinct cell-specific control of autoimmunity and infection by FcgammaRIIb. J Exp Med. 2008;205:883–95.

Catalan D, Aravena O, Sabugo F, Wurmann P, Soto L, Kalergis AM, Cuchacovich M, Aguillon JC. B cells from rheumatoid arthritis patients show important alterations in the expression of CD86 and FcgammaRIIb, which are modulated by anti-tumor necrosis factor therapy. Arthritis Res Ther. 2010;12:R68.

Foy TM, Lynch RG, Waldschmidt TJ. Ontogeny and distribution of the murine B cell Fc gamma RII. J Immunol. 1992;149:1516–23.

Fukuyama H, Nimmerjahn F, Ravetch JV. The inhibitory Fcgamma receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G+ anti-DNA plasma cells. Nat Immunol. 2005;6:99–106.

Isaak A, Gergely Jr P, Szekeres Z, Prechl J, Poor G, Erdei A, Gergely J. Physiological up-regulation of inhibitory receptors Fc gamma RII and CR1 on memory B cells is lacking in SLE patients. Int Immunol. 2008;20:185–92.

Jiang Y, Hirose S, Sanokawa-Akakura R, Abe M, Mi X, Li N, Miura Y, Shirai J, Zhang D, Hamano Y, Shirai T. Genetically determined aberrant down-regulation of FcgammaRIIB1 in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. Int Immunol. 1999;11:1685–91.

Mackay M, Stanevsky A, Wang T, Aranow C, Li M, Koenig S, Ravetch JV, Diamond B. Selective dysregulation of the FcgammaIIB receptor on memory B cells in SLE. J Exp Med. 2006;203:2157–64.

Nimmerjahn F, Ravetch JV. Antibody-mediated modulation of immune responses. Immunol Rev. 2010;236:265–75.

Paul E, Nelde A, Verschoor A, Carroll MC. Follicular exclusion of autoreactive B cells requires FcgammaRIIb. Int Immunol. 2007;19:365–73.

Rahman ZS, Alabyev B, Manser T. FcgammaRIIB regulates autoreactive primary antibody-forming cell, but not germinal center B cell, activity. J Immunol. 2007;178:897–907.

Ravetch JV, Nimmerjahn F. Fc receptors and their role in immune regulation and inflammation. In: Paul WE, editor. Fundamental immunology. 6th ed. Philadelphia: Wolters Kluwer; 2008. p. 684–705.

Rudge EU, Cutler AJ, Pritchard NR, Smith KG. Interleukin 4 reduces expression of inhibitory receptors on B cells and abolishes CD22 and Fc gamma RII-mediated B cell suppression. J Exp Med. 2002;195:1079–85.

Su K, Yang H, Li X, Gibson AW, Cafardi JM, Zhou T, Edberg JC, Kimberly RP. Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. J Immunol. 2007;178:3272–80.

Tackenberg B, Jelcic I, Baerenwaldt A, Oertel WH, Sommer N, Nimmerjahn F, Lunemann JD. Impaired inhibitory Fcgamma receptor IIB expression on B cells in chronic inflammatory demyelinating polyneuropathy. Proc Natl Acad Sci USA. 2009;106:4788–92.

Tackenberg B, Nimmerjahn F, Lunemann JD. Mechanisms of IVIG efficacy in chronic inflammatory demyelinating polyneuropathy. J Clin Immunol. 2010;1:S65–9.

Tarasenko T, Dean JA, Bolland S. FcgammaRIIB as a modulator of autoimmune disease susceptibility. Autoimmunity. 2007;40:409–17.

Wijngaarden S, van de Winkel JG, Jacobs KM, Bijlsma JW, Lafeber FP, van Roon JA. A shift in the balance of inhibitory and activating Fcgamma receptors on monocytes toward the inhibitory Fcgamma receptor

IIb is associated with prevention of monocyte activation in rheumatoid arthritis. Arthritis Rheum. 2004;50:3878–87.
Xiang Z, Cutler AJ, Brownlie RJ, Fairfax K, Lawlor KE, Severinson E, Walker EU, Manz RA, Tarlinton DM, Smith KG. FcgammaRIIb controls bone marrow plasma cell persistence and apoptosis. Nat Immunol. 2007;8:419–29.

Fibrosis

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Synonyms

Collagen; Extracellular matrix; Fibroblasts; Myofibroblast; Scar; TGF-β

Definition

Fibrosis is the deposition of excess fibrous connective tissue replacing normal tissue architecture. Connective tissue cells synthesize and maintain extracellular matrix (ECM) and provide mechanical support and attachment for contiguous tissues and organs. The cells embedded within connective tissues live in specialized environments in which they experience tissue-specific chemical and mechanical signals. For all connective tissues, collagens are the principal molecular building blocks. Collagens control tissue architecture, tensile strength, and cell–matrix and matrix–matrix interactions. The ECM is the main source of collagens, other macromolecules such as proteoglycans, fibronectin, fibrillins, as well as adhesion molecules, growth factors, and other signaling molecules. Exaggerated deposition and disordered remodeling of ECM characterize the fibrotic process (Hynes 2009; Chan et al. 2010).

Fibrosis is not always pathological; the fibrotic process is a key event underlying normal wound healing and tissue repair. The level of collagen accumulation during healing is tightly regulated by a balance between synthesis and degradation. The fibroblast is the principal cell type that both synthesizes and degrades ECM components, orchestrated by chemical and biomechanical signals in endocrine, paracrine, and autocrine fashion.

Fibroblasts are mesenchymal cells identified by their location and stellate morphology, and by the absence of specific surface markers. They are found in most parenchymal tissues, especially those with prominent epithelial and microvascular components (e.g., skin, lung, liver, and kidney). Fibroblasts are important in normal development, tissue homeostasis, and wound healing. Injury and repair of most tissues begin with epithelial or endothelial damage, resulting in exudation of blood or platelet-rich plasma into extravascular spaces, leading to the formation of a fibrinous clot (provisional matrix). Fibroblasts migrate into the provisional matrix where they proliferate and produce de novo ECM components, creating a fibroblast-populated granulation tissue. Although fibroblast activation during wound healing is beneficial in rebuilding the ECM, excess collagen deposition results in pathological fibrosis and scar formation. The disordered and exaggerated deposition of ECM in affected tissues disrupts the normal architecture of the affected organs, leading to their dysfunction and ultimately failure. Therefore, fibrosis represents an uncontrolled repair process that recapitulates features of embryonic development and normal wound healing. This scenario posits that fibrosis and scarring results from failure to stop tissue remodeling rather than a specific continuing insult. Normally, the healing process is turned on and then turned off in a temporally and spatially controlled manner. In the absence of approximately timed off signals, there is an increase in cell proliferation, matrix accumulation, and remodeling, resulting in a non-resolved and intractable fibrotic scar (Wynn 2008; Rosenbloom et al. 2010; Wei et al. 2011).

It is estimated that fibrotic conditions account for up to 45 % of deaths in the developed

Fibrosis, Table 1 Selected organ-specific fibrosing conditions

• **Skin**
Hypertrophic scar
Morphea
Nephrogenic fibrosing dermatopathy (may be systemic)
Dupuytren contracture
Eosinophilic fasciitis
• **Lung**
Idiopathic pulmonary fibrosis
Sarcoidosis
Radiation pneumonitis
• **Liver**
Cirrhosis (alcohol, hepatitis, schistosomal, cryptogenic)
Primary biliary cirrhosis
• **Kidney**
Glomerulosclerosis
• **Heart**
Hypertrophic and dilated cardiomyopathy
Endomyocardial fibrosis
• **Multisystemic diseases**
Systemic sclerosis
Sclerodermatous graft versus host disease

world (Wynn 2008; Bhattacharyya et al. 2011). They encompass a wide spectrum of clinical entities, including organ-specific disorders in the skin, lung, liver, kidney, and heart, as well as multisystemic diseases such as systemic sclerosis (Table 1). Although their etiology, causative mechanisms, and underlying genetic factors are different, these conditions appear to share common feature of disordered and exaggerated deposition of ECM in affected tissues (Wynn 2008).

There are no approved anti-fibrotic therapies. Fibrosis thus represents an unmet medical need. Developing effective anti-fibrotic therapies remains a major challenge given the plethora of molecules contributing to this process, and the complex networks linking them.

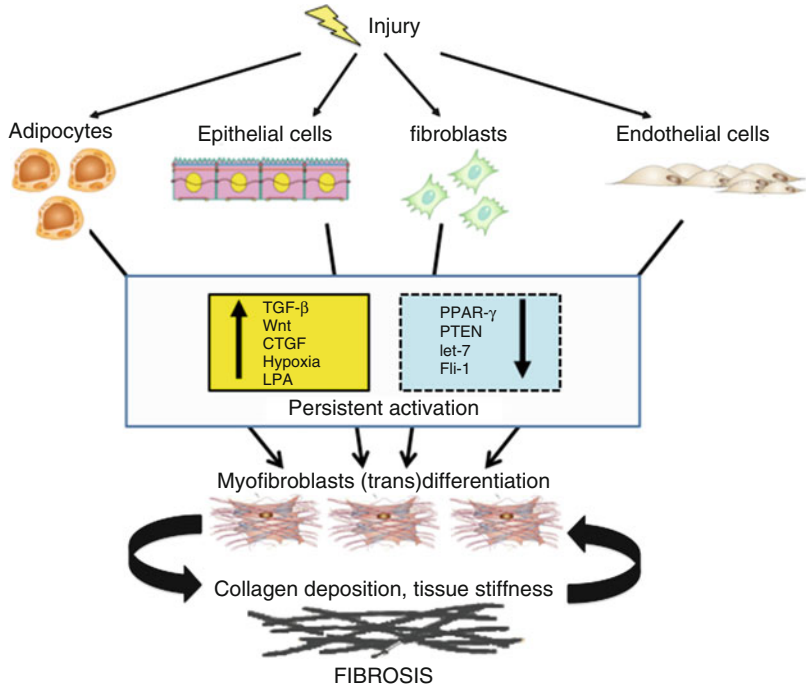
Mechanisms That Promote Fibrosis

During the fibrotic process, fibroblasts acquire a myofibroblastic phenotype. Myofibroblasts express α-smooth muscle actin (α-SMA) in stress fibers, which promotes strong force generation. During normal tissue repair, transient myofibroblast accumulation is important for restoring tissue integrity by forming a mechanically resistant scar. However, in fibrotic diseases, myofibroblasts persist in the lesion, leading to tissue deformations. The widely used molecular identifier of the myofibroblast is α-SMA. The main cues for myofibroblast differentiation and persistence are mechanical forces generated from the ECM and transforming growth factor-β (TGF-β). Tissue stiffness is increased in fibrotic organs and tissues and contributes directly to fibroblast activation (Hinz et al. 2012).

Myofibroblasts originate from resident mesenchymal fibroblasts and from circulating fibroblast-like cells (fibrocytes) derived from bone marrow stem cells. Myofibroblasts may also originate via the transdifferentiation of non-mesenchymal lineages (such as epithelial cells, pericytes, endothelial cells, and adipocytes), although the role of cellular plasticity in the pathogenesis of fibrosis remains controversial (Zeisberg and Neilson 2009; Hinz et al. in press).

Epithelial–mesenchymal transition (EMT) is important during embryogenesis and in renal, pulmonary, and liver fibrosis (Zeisberg and Neilson 2009). Endothelial–mesenchymal transition (EndoMT), characterized by loss of endothelial cell markers and acquisition of a profibrogenic phenotype, can be induced by TGF-β, hypoxia, Wnt ligands, Hedgehog, Jagged–Notch signaling, and bioactive lipids (Fig. 1) (Bhattacharyya et al. 2011; Piera-Velazquez et al. 2011).

The ubiquitously expressed and multifunctional cytokine TGF-β is recognized as a master regulator of both normal wound healing and pathological fibrosis. TGF-β regulates the growth, differentiation, survival, and function of various cell types. In the fibrotic cellular environment, the bioavailability of TGF-β is regulated both by its secretion from tissue-infiltrating macrophages and by local activation of its matrix-bound latent form. Latent TGF-β activation is mediated by cell-surface integrins such as $\alpha_v\beta_6$ on epithelial cells or fibroblasts that cause conformation changes in TGF-β resulting in liberation of the active form (Shi et al. 2011). The profibrotic cellular responses triggered by TGF-β are mediated via both canonical



Fibrosis, Fig. 1 After injury, a variety of cells can be activated including adipocytes, fibroblasts, and epithelial and endothelial cells. Elevated profibrotic signaling and/or repressed anti-fibrotic signaling leads to myofibroblasts differentiation and activation and eventually collagen deposition to restore tissue integrity. The persistence of myofibroblasts represents a deregulated and uncontrolled repair process and results in a non-resolved fibrotic wound with exaggerated deposition of collagen in affected tissues

Smad and Smad-independent signal transduction pathways involving the c-abl, ERK1/2, PI3K/AKT, FAK1, AKT, JNK, and p38. TGF-β initiates signaling through the ligand-dependent activation of a complex consisting of TGF-β receptor type I and II, and the signal is transmitted to the nucleus by Smad proteins (canonical Smad). The activated TGF-βRI directly phosphorylates cytosolic Smad2 and Smad3, which translocate to the nucleus as a heterocomplex with Smad4. Smad generally require interaction with other DNA-binding partners and coactivators such as Sp1, CREB-binding protein (CBP)/p300, and AP-1. Ligand-induced Smad signal transduction is tightly controlled by endogenous inhibitors such as Smad7, bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI), and Nab-2, which are themselves regulated by TGF-β in negative feedback loops. Although pharmacological blockade of TGF-β is feasible as a strategy for controlling fibrosis, the exceptionally pleiotropic actions of TGF-β raise concerns that interfering with this essential physiological signaling molecule could result in a broad range of toxicities such as autoimmunity and defective tissue repair (Jinnin 2010; Bhattacharyya et al. 2011; Wei et al. 2011).

The Wnts are a family of poorly soluble secreted glycoproteins that regulate both cell – cell adhesion and gene transcription. Wnts have a key role in embryonic development and cell fate specification. Intracellular Wnt signaling is mediated via canonical (β-catenin-dependent) and noncanonical pathways, which engage in extensive cross talk with TGF-β signaling. The stimulation of normal fibroblasts with Wnt ligands results in β-catenin-mediated enhancement of collagen and matrix gene expressions, enhanced myofibroblast differentiation and increased cell migration. Chronic hyperactivation of β-catenin signaling in fibrosis represents aberrant recapitulation of embryologic developmental programs. It might result from Wnt-ligand-independent, constitutive β-catenin activation induced by ECM components such as fibronectin-extra domain A (EDA) and tenascin acting in a self-amplifying autocrine feedback loop (Bhattacharyya et al. 2011).

Connective tissue growth factor (CTGF), a member of the CTGF/Cyr61/NOV (CCN)

family of matricellular proteins, is induced substantially by TGF-β and is implicated in fibrogenesis. CTGF is overexpressed in most fibrotic diseases. It is thought that CTGF may, in concert with TGF-β, exert a variety of fibrotic activities although the mechanisms involved are still controversial.

Bioactive lipids are potent modulators of fibroblast function. Lysophosphatidic acid (LPA; l-acyl-2-hydroxy-*sn*-glycero-3-phosphate) is a growth-factor-like mediator acting on G-protein-coupled receptors. LPA is detectable in biological fluids including serum and saliva. Increased production of LPA in fibrotic diseases stimulates fibroblast proliferation and migration, enhances CTGF expression, induces myofibroblast transdifferentiation, and augments TGF-β signaling. Interestingly, LPA-null mice exhibit attenuated fibrotic responses.

Toll-like receptors (TLRs) are sensors of the innate immune system that allow recognition of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), resulting in initiation of immune responses. DAMPs are generated at sites of tissue injury in response to mechanical damage, inflammation, and oxidative stress. Increased expression of TLR4 has been described in various forms of fibrosis. Fibroblast TLR signaling initiated by damage-associated endogenous TLR ligands such as fibronectin-EDA or hyaluronic acid might act as the "switch" to convert a self-limited physiological regenerative tissue repair process into an aberrant fibrotic scar.

Mechanisms That Inhibit Fibrosis

The persistence and progression of pathological fibrosis is attributed in part to the failure of intrinsic anti-fibrotic mechanisms that normally restrain fibroblast activation. An important component of innate anti-fibrotic defense is peroxisome proliferator-activated receptor gamma (PPAR-γ). Originally identified in adipose tissue, PPAR-γ is a dual-function molecule acting both as a nuclear receptor and ligand-inducible transcription factor. PPAR-γ modulates metabolic, vascular, and immune processes, and abnormal PPAR-γ function is implicated in lipodystrophy, atherosclerosis, pulmonary artery hypertension, cancer, and inflammatory diseases. Fibrosis is associated with defective expression or function of PPAR-γ in the lesional tissues (Wei et al. 2010). PPAR-γ expression in lesional tissue shows an inverse relationship with enhanced TGF-β signaling (Wei et al. 2011). Other innate anti-fibrotic mechanisms include phosphatase and tensin homolog (PTEN), Ski/Sno, and Fli-1 (Bhattacharyya et al. 2011).

miRNAs are small noncoding ribonucleic acids (RNAs) that contribute to fibroblast activation and fibrosis. Expression of miR-29, an anti-fibrotic microRNA, is markedly reduced in fibrotic tissues. Another miRNA, miR-21, is upregulated in lung, heart, and renal fibrosis and enhances fibrogenic activity. In contrast, let-7d is downregulated by TGF-β, and its loss leads to a gain of a profibrotic phenotype.

Organ-Specific Fibrosis

Pulmonary fibrosis represents a heterogeneous group of lung disorders characterized by progressive destruction of lung architecture caused by scar formation that leads to respiratory failure. Pulmonary fibrosis can be idiopathic or can develop following viral infections, bone marrow transplantation, radiotherapy, chemotherapeutic drugs, environmental toxins, and chronic inflammatory disease such as systemic sclerosis. Idiopathic pulmonary fibrosis affects more than 50,000 people in the United States and is associated with poor survival.

Despite improvements in diagnosis, pulmonary fibrosis remains refractory to treatment, and lung transplantation is the only effective treatment currently available.

Recurrent epithelial injury is considered to be the primary defect in idiopathic pulmonary fibrosis, and inflammation is variable. Ultrastructural, immunohistochemical, and bronchoalveolar lavage studies suggest alveolar type II cell injury as an early feature. The hypothesis of epithelial cell death as a driver for pulmonary fibrosis is

hampered by recent findings of mutations in the genes for surfactant proteins and genes that maintain telomere length. In addition, studies using the bleomycin model of lung injury suggest that inhibition of epithelial cell apoptosis attenuates fibrosis. While the "inflammatory" concept of pulmonary fibrosis dominated the field in the past, precise histologic definition of the disease has made it clear that inflammation in the lung is relatively slight even in the earliest phase of the disease. Furthermore, there is a lack of correlation of inflammatory markers with disease progression, and conventional immunosuppressive agents fail to arrest or reverse lung fibrosis (Homer et al. 2011; Wynn 2011).

The loss of basement membrane integrity is an important factor for aberrant lung remodeling. Interaction of epithelial cells with an abnormal ECM in the presence of active TGF-β1 leads to EMT and collagen accumulation (Wynn 2011).

Coagulation cascade activation has also been implicated as an important and early event following lung injury. The serine protease thrombin plays a major role by promoting the myofibroblast differentiation of lung fibroblasts and induction of fibrogenic cytokines and ECM proteins, contributing to progression of pulmonary fibrosis.

Liver Fibrosis

Liver architecture suffers transient changes after an acute or self-limited injury. However, if the injury is sustained, chronic inflammation and ECM accumulation persist, leading to fibrosis and cirrhosis, characterized by a distortion of the liver parenchyma and vascular architecture. Progression from liver injury to cirrhosis is generally slow, largely asymptomatic, and influenced by both genetic and environmental factors (Hernandez-Gea and Friedman 2011).

Hepatic fibrosis develops as a result of the progressive thickening of fibrotic septa and increasing cross-linking of collagen, disrupting the tightly organized liver parenchyma that provides functional and structural integrity. The hepatic ECM normally comprises less than 3 % of the relative area on a liver tissue section and approximately 0.5 % of the wet weight (Hernandez-Gea and Friedman 2011).

Hepatic scarring is a common pathway resulting from toxic, metabolic, and infectious insults. Activated hepatic stellate cells are key effectors of liver fibrosis. Following liver injury, hepatic stellate cells become activated, with loss of vitamin A droplets and acquisition of myofibroblast features (Friedman 2010; Hernandez-Gea and Friedman 2011).

PPAR-γ is expressed in unstimulated hepatic stellate cells and plays an important role in maintaining their quiescence. During liver injury, PPAR-γ expression is reduced, contributing to hepatic stellate cell activation. Other cells contributing to the fibrotic process in the liver include portal fibroblasts in biliary diseases such as ischemia and cholestasis. Fibrocytes and EMT have also been reported to play a role in liver regeneration and fibrosis (Hernandez-Gea and Friedman 2011).

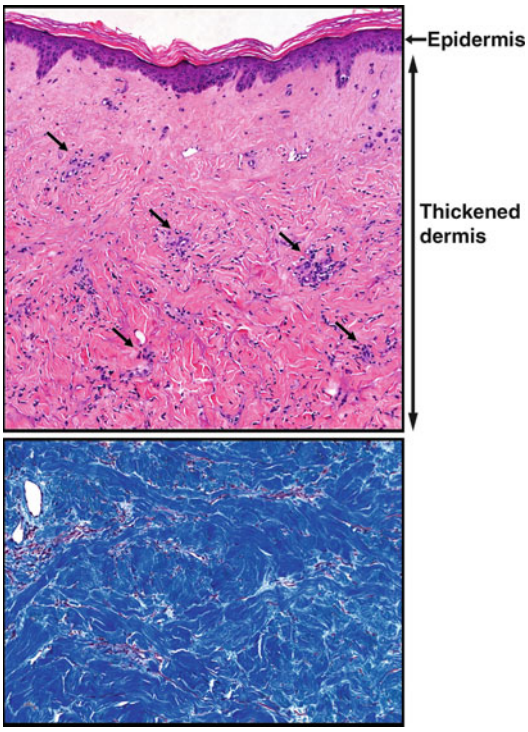
Resident hepatic macrophages called Kupffer cells participate in both progression and regression of liver fibrosis. Kupffer cells are the first point of contact for bacterial products and endotoxin (LPS) derived from the gastrointestinal tract. Through Toll-like receptor 4, the TGF-β pseudoreceptor BAMBI is downregulated in hepatic stellate cells, thereby enhancing TGF-β signaling and hepatic fibrogenesis. Interestingly, single-nucleotide polymorphisms of the *TLR* gene (D299G and T399I) associated with reduced LPS responsiveness confer a significantly reduced risk for fibrosis progression in patients with chronic hepatitis C infection (Hernandez-Gea and Friedman 2011).

Kidney Fibrosis

Renal fibrosis underlies the progression of chronic kidney disease to end-stage renal disease. Renal fibrosis results from inflammatory or immunological (pyelonephritis and lupus nephritis), obstructive (kidney stones), metabolic (diabetic nephropathy), or systemic (nephrogenic systemic fibrosis) injury ultimately progressing

to end-stage renal disease. Intrarenal hypertension and hyperfiltration, oxidative stress, angiotensin II, leptin, inflammatory cytokines, hyperinsulinemia/insulin resistance, hyperglycemia, and impaired lipid metabolism all contribute to renal fibrosis. The histologic features of renal fibrosis encompass the progressive appearance of glomerulosclerosis, tubulointerstitial fibrosis, and changes in renal vasculature (loss of glomerular and peritubular capillaries). The key events of tubulointerstitial fibrosis are initiated by mesangial cell activation and EMT of the podocytes.

Nephrogenic systemic fibrosis is a recently recognized fibrotic condition in patients with chronic renal disease. It is thought to be triggered by exposure to gadolinium, a contrast agent used for magnetic resonance imaging. Patients develop diffuse scleromyxedema-like skin lesions and less commonly, systemic manifestations with fibrosis in the lungs, myocardium, and skeletal muscles. The pathogenesis of this disorder may involve gadolinium activation of dendritic cells via TLR and accumulation of circulating fibrocytes. Chelated gadolinium directly stimulates macrophages and monocytes to release profibrotic cytokines and growth factors capable of inducing tissue fibrosis (Del Galdo et al. 2010).



Fibrosis, Fig. 2 Skin fibrosis in Scleroderma. (**a**) The papillary and reticular dermis is filled with markedly thickened collagen fibers. Lymphocytic perivascular infiltrate are present (arrows). Note loss of hair follicles and glandular structures. Hematoxylin–eosin-stained section, optical micrograph, original magnification X 100. (**b**) Thickened collagen bundles stained deep blue with Masson's trichrome stain, original magnification X 100

Systemic Sclerosis

Systemic sclerosis (SSc) is a chronic autoimmune disease with multiorgan fibrosis, associated with immune dysregulation and obliterative microvasculopathy (Fig. 2). Systemic sclerosis has multiple clinical subsets that are defined in part based on to the extent of skin involvement. It has one of the highest mortality rates among rheumatic diseases and lacks effective disease-modifying therapies. Lung involvement is the leading cause of death in patients with SSc (Bhattacharyya et al. 2011).

Although the cause of SSc is not known, its clinical and pathological manifestations are result from a complex interaction between genetic factors and environment. Genetic polymorphisms associated with SSc include genes involved in immune regulation such as BANK1, C8orf13-BLK, IL-23R, IRF5, STAT4, CD247, TBX21, and TNFSF422 as well as TGF-βRI and PPAR-Y. However, the most significant genetic association is with HLA types and defined autoantibodies (Bhattacharyya et al. 2011).

The pathogenesis of fibrosis in SSc has been investigated extensively. The "SSc fibroblast phenotype" is characterized by constitutively enhanced ECM synthesis, secretion of profibrotic cytokines and chemokines, resistance to INF-gamma and other inhibitory signals, and spontaneous generation of reactive oxygen species. The SSc fibroblast phenotype could represent a

cell-autonomous abnormality, perhaps due to epigenetic modifications, or activation in response to exogenous stimuli in the fibrotic milieu.

Fibroblasts explanted from lesional fibrotic tissue of SSc patients display phenotypic alterations that persist after several passages in vitro. One of the factors responsible for the induction and maintenance of this phenotype is autocrine TGF-β activation. Of note, SSc fibroblast characteristics can be induced in normal fibroblasts by treatment with TGF-β. Consistent with this hypothesis, SSc fibroblasts show elevated Smad3 activation and constitutive Smad interaction with the coactivator histone acetyltransferase p300/CBP. Molecules involved in intracellular signaling are abnormally expressed or constitutively activated in SSc fibroblasts in culture, including protein kinase C, Smad3, Smad7, Egr-1, p300, and c-Abl, as well as several microRNA species. Furthermore, defective expression and function of endogenous suppressors of TGF-β signaling and ECM production such as Smad7, BAMBI, and miR29 have also been demonstrated, suggesting that failure to restrain fibroblast activation may represent a fundamental defect in SSc (Jinnin 2010; Bhattacharyya et al. 2011; Wei et al. 2011).

Fibrosis Treatment

The fibrotic process causes progressive and ultimately irreversible loss of functional integrity of vital organs. Fibrosis is traditionally considered to be an intractable process, and when it affects vital organs, it is associated with high mortality. Cellular plasticity, aberrant recapitulation of embryological genetic programs, altered epigenetic modifications and miRNA regulation, and the occurrence of self-amplifying feed-forward loops driven by hypoxia, matrix stiffness, and tissue damage all contribute to uncontrolled fibroblast activation that results in an abnormal tissue repair process, progressing to intractable fibrosis.

Many potential opportunities exist for interfering with the fibrotic process, and several existing drugs approved for other indications show an anti-fibrotic effect. Ultimately, effective therapeutic strategies for fibrotic diseases will be designed to block converging fibrogenic pathways at multiple levels (Table 2).

Fibrosis, Table 2 Potential targets for anti-fibrotic therapy

Profibrotic mediators
Growth factors:
Transforming growth factor-β
CCN family
Wnt-β-catenin pathway
Integrins: $\alpha_v\beta_6$
Clotting cascade: thrombin
Bioactive lipids: lysophosphatidic acid (LPA)
Oxidative stress: reactive oxygen species (ROS)
Innate immunity: Toll-like receptors (TLR4)
Cartilage oligomeric matrix protein
Endoplasmic reticulum stress
Anti-fibrotic agonists
Type I interferon
Peroxisome proliferator-activated receptors
NF-E2-related factor 2 (Nrf-2)

Cross-References

- ▶ Environment and autoimmunity
- ▶ Sarcoidosis
- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Scleroderma-Like Conditions of the Skin
- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Scleroderma: Genetics
- ▶ TGF-β
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. Nat Rev Rheumatol. 2011;8(1):42–54.

Chan MW, Hinz B, McCulloch CA. Mechanical induction of gene expression in connective tissue cells. Methods Cell Biol. 2010;98:178–205.

Del Galdo F, Shaw MA, Jimenez SA. Proteomic analysis identification of a pattern of shared alterations in the secretome of dermal fibroblasts from systemic sclerosis and nephrogenic systemic fibrosis. Am J Pathol. 2010;177:1638–46.

Friedman SL. Evolving challenges in hepatic fibrosis. Nat Rev Gastroenterol Hepatol. 2010;7:425–36.

Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol. 2011;6:425–56.

Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmoulière A, Varga J, De Wever O, Mareel M, Gabbiani G. Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. Am J Pathol. 2012;18:1340–55.

Homer RJ, Elias JA, Lee CG, Herzog E. Modern concepts on the role of inflammation in pulmonary fibrosis. Arch Pathol Lab Med. 2011;135:780–8.

Hynes RO. The extracellular matrix: not just pretty fibrils. Science. 2009;326:1216–9.

Jinnin M. Mechanisms of skin fibrosis in systemic sclerosis. J Dermatol. 2010;37:11–25.

Piera-Velazquez S, Li Z, Jimenez SA. Role of endothelial-mesenchymal transition (EndoMT) in the pathogenesis of fibrotic disorders. Am J Pathol. 2011;179:1074–80.

Rosenbloom J, Castro SV, Jimenez SA. Narrative review: fibrotic diseases: cellular and molecular mechanisms and novel therapies. Ann Intern Med. 2010;152:159–66.

Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, Springer TA. Latent TGF-beta structure and activation. Nature. 2011;474:343–9.

Wei J, Bhattacharyya S, Tourtellotte WG, Varga J. Fibrosis in systemic sclerosis: emerging concepts and implications for targeted therapy. Autoimmun Rev. 2011;10:267–75.

Wei J, Ghosh AK, Sargent JL, Komura K, Wu M, Huang QQ, Jain M, Whitfield ML, Feghali-Bostwick C, Varga J. PPARgamma downregulation by TGFss in fibroblast and impaired expression and function in systemic sclerosis: a novel mechanism for progressive fibrogenesis. PLoS One. 2010;5:e13778.

Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol. 2008;214:199–210.

Wynn TA. Integrating mechanisms of pulmonary fibrosis. J Exp Med. 2011;208:1339–50.

Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest. 2009;119:1429–37.

G

Genetics of Juvenile Idiopathic Arthritis

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Synonyms

Genetics of juvenile arthritis syndromes

Definition

Juvenile idiopathic arthritis (JIA) encompasses chronic childhood arthritis of unknown etiology and is manifest by diverse clinical symptoms and outcomes. Patterns of inheritance consistent with Mendelian or monogenic inheritance have not been observed in JIA. Although familial history of JIA is sparse, a familial history of autoimmunity is observed in 13–15 % of families with JIA probands, whereas only 4 % of the control families are afflicted with autoimmune disease. JIA is thought to result from interactions among genetic factors, immune mechanisms, and environmental exposures. Most of the genetic predisposition to JIA is determined by the major histocompatibility complex (MHC) loci. Multiple MHC loci are associated with the development of JIA, with specific disease subtypes being linked to specific human leukocyte antigen (HLA) alleles. In general, these associations are distinct from those in adult rheumatoid arthritis. The spectrum of non-HLA genes that may be associated with JIA is expanding rapidly, but their contribution to the risk of JIA appears to be less significant, and their effects may not be specific to JIA.

Introduction

Juvenile idiopathic arthritis encompasses chronic childhood arthritis of unknown etiology and is manifest by diverse clinical symptoms and outcomes (Petty et al. 1998). The relatively new term "*Juvenile idiopathic arthritis* (*JIA*)" has replaced the older terms "*juvenile rheumatoid arthritis*" (previously used commonly in the United States) and "*juvenile chronic arthritis*" (previously preferred in Europe). Currently, *JIA* incorporates all of what was called *juvenile rheumatoid arthritis* in the past, and also includes all other forms of "idiopathic" arthritis in childhood. Currently, six subtypes of JIA are distinguished largely on the basis of clinical and laboratory features present in the first 6 months of disease, with a seventh category reserved for individuals who cannot be unambiguously classified. Thus, patients with **persistent oligoarthritis** have cumulative involvement of fewer than five joints, whereas **extended oligoarthritis** indicates involvement of five or more joints sometime after 6 months of disease. **Polyarthritis** involves

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five or more joints within the first 6 months of disease and is subdivided by the presence or absence of rheumatoid factor (**RF-positive** or **RF-negative polyarthritis**). **Enthesitis-related arthritis** (ERA) typically affects older (>6 years) males who frequently have HLA-B27 and may have a family history of spondyloarthropathy. **Psoriatic arthritis** is usually associated with typical psoriatic skin lesions in the patients themselves or their first-degree relatives. **Systemic JIA** involves chronic arthritis and associated systemic features that may include quotidian fevers, erythematous rash, generalized lymphadenopathy, and hepatosplenomegaly. Although this new JIA classification has increased phenotypic homogeneity within some subtypes, there is still significant heterogeneity within almost each of the JIA subtypes as well as some commonalities between subtypes.

The pathogenesis and etiology of JIA are unclear. As with most autoimmune disorders (see ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis), JIA is thought to result from interactions among genetic factors, immune mechanisms, and environmental exposures. Due to high clinical heterogeneity, our understanding of the genetic factors involved in JIA has not progressed as quickly as compared with other autoimmune arthropathies, including rheumatoid arthritis and ankylosing spondylitis. The necessity to split the JIA patient population into smaller groups provides challenges for recruiting patient cohorts of adequate size with sufficient analytical power for genomic studies. In addition, the shift from the old *JRA* to the new *JIA* classification system often makes it difficult to directly compare the results between more recent and older studies.

Familial Predisposition

Patterns of inheritance consistent with Mendelian or monogenic inheritance have not been observed in JIA. The prevalence of JIA among siblings of probands is about 15–30 times greater than the population prevalence of approximately 1 in 1,000 children (Towner et al. 1983; Clemens et al. 1985). Twin studies have shown that the monozygotic twin concordance rates for JIA in general range between 25 % and 40 %. Families with multiple affected members are rare (Clemens et al. 1985; Rossen et al. 1980). In the United States, the JRA Affected Sib Pairs Research Registry in Cincinnati estimated the total number of affected sib pairs at 300 in a population of approximately 250 million (Moroldo et al. 1997, 2004). Almost 80 % of the affected pairs and 100 % of twin pairs were concordant for disease type. Furthermore, siblings were more likely to develop JIA at the same age of onset, rather than the same calendar year. Among twins, disease onset was separated by a mean of approximately 3 months.

In contrast to the sparse familial history of JIA, a familial history of autoimmunity is observed in 13–15 % of families with JIA probands, whereas only 4 % of the control families are afflicted with autoimmune disease (Prahalad et al. 2002). These observations are consistent with the idea that while some genetic associations are unique to JIA, others overlap with other autoimmune diseases.

Predisposing MHC Genes

Predisposing MHC genes are the best characterized genetic factors in JIA. Multiple HLA loci are associated with the development of JIA, with specific disease subtypes being linked to specific HLA alleles (Rossen et al. 1980; Nepom and Glass 1992). In general, these associations are distinct from those in adult rheumatoid arthritis (Stastny and Fink 1979) (see ▶ Rheumatoid Arthritis, Genetics). Genes that do not encode classic HLA proteins, but lie within the major histocompatibility complex (MHC), may also predispose to JIA.

Early-onset oligoarticular JIA – Oligoarticular JIA patients with early onset of the disease (i.e., before 6 years of age) constitute the subtype with the most distinctive HLA associations. Interestingly, this subtype of JIA has a relatively high concordance among siblings (Moroldo et al. 2004). The early reports of associations with various HLA class II DR and DP

serologic specificities including HLA-DR8 (Stastny and Fink 1979; Van Kerckhove et al. 1990), HLA-DR5 (DR11) (Glass et al. 1980; Førre et al. 1983), HLA-DR13 (Reekers et al. 1983), and HLA-DPw2 (Hoffman et al. 1986; Odum et al. 1986) have been confirmed by DNA-sequence-dependent methodologies. In oligoarticular JIA, disease susceptibility correlates with particular alleles of each serologically defined specificity, such as HLA-DRB1*0801 of DR8 and HLA-DPB1*0201 of DPw2 (Nepom and Glass 1992). Although most associations are with HLA class II, a consistent increase in risk with the HLA class I A2 allele has been reported in girls with early-onset oligoarticular JIA as well (Oen et al. 1982). In contrast to these findings, some HLA class II alleles occur with decreased frequency in this group of children, suggesting a protective effect. Examples include HLA-DR4 and HLA-DR7 (Nepom and Glass 1992).

Determination of HLA specificities using DNA-based methodologies has also better defined the genetic markers of disease outcome in early-onset oligoarticular JIA. Haplotypes carrying HLA-DRB*0801, HLA-DRB*1301, and HLA-DP*0201 alleles appear to predispose to oligoarticular JIA in general. In contrast, other alleles may contribute to the risk for other clinical manifestations. For example, HLA-DRB1*1104 predisposes to eye disease (i.e., chronic anterior uveitis) (Melin-Aldana et al. 1992), while HLA-DQA1*0101 is associated with a reduced risk of eye disease, but predisposes to evolution from oligoarticular to more aggressive polyarticular disease (van Kerckhove et al. 1991). HLA-DRB1*0101 has also been associated with polyarticular disease (Ploski et al. 1993). Whether the HLA-DQA*0101 or the HLA-DRB*0101 allele is a better marker for the haplotype is uncertain, but there are data that are compatible with either possibility.

The fact that HLA genes from four loci (HLA-A, HLA-DR/DQ, and HLA-DP) are involved in inherited predisposition to oligoarticular JIA raises the possibility that these genes make up a potential susceptibility haplotype. However, the absence of linkage disequilibrium between these three loci indicates that there are independent genetic effects. In a significant proportion of patients with early-onset pauciarticular JIA, especially those with uveitis, the development of the disease may be associated with the presence of two susceptibility alleles of either the HLA-DR or HLA-DQ loci. Interestingly, the cumulative addition of risk factors leads to increasing odds ratios. For instance, patients who are heterozygous for HLA-DR5/HLA-DR8 have a particularly increased risk for developing eye disease (Hall et al. 1986). An HLA-DP gene and the class I HLA-A2 gene also contribute, appearing as independent risk factors. Another recent study demonstrated that the number of susceptibility alleles was inversely proportional to the age of onset of JIA. Among children who carried HLA-A2, DPB1*0201, and one HLA-DR susceptibility allele, the median age of onset of oligoarticular JIA was just 2.4 years (Murray et al. 1999). Remarkably, HLA Class II gene homozygosity is not increased in the oligoarticular JIA patient population, suggesting that a dosage effect of any individual gene does not increase risk (Hall et al. 1986).

Other JIA subtypes. HLA associations with clinical forms other than early-onset oligoarticular JIA are less strong. As previously mentioned increased risk of **enthesitis-related JIA** is associated with HLA-B27 (Rachelefsky et al. 1974; Schaller et al. 1976). **Polyarticular onset, rheumatoid factor (RF)-negative JIA** has been associated with HLA-DPB1*03 (Fernandez-Viña et al. 1990). Similarly to early-onset oligoarticular JIA, JIA patients with early-onset polyarticular disease also show an association with HLA-DRB*0801. **RF-positive polyarticular JIA** has the same HLA-DR4 associations as does adult RA: HLA-DRB1*0401 and HLA-DRB1*0101 (Nepom et al. 1984). In systemic JIA, only a weak association was found with the 3 Mb interval that contains the *HLA-DRB1* and *HLA-DQA1* genes (Ombrello et al. 2011).

HLA-DR1/DR4 shared epitope hypothesis – Rheumatoid arthritis in adults is associated with the presence of the DNA sequence common to

HLA-DR1 and some HLA-DR4 specificities (Gregersen et al. 1987) (see ▶ Rheumatoid Arthritis, Genetics). However, the shared epitope hypothesis as applied to adults is not useful for oligoarticular JIA, in which HLA-DR4 is protective and HLA-DR1 haplotypes predispose to a polyarticular outcome. Similarly, patients with seronegative polyarticular JIA do not carry the appropriate epitopes. The only JIA group in which the shared epitope hypothesis may hold is in older children with polyarticular disease who are IgM RF-positive. These patients have the childhood equivalent of adult RA; they represent fewer than 10 % of all patients with JIA.

Age-specific effects – There is new evidence that some genes operative in JIA appear to have a "window-of-effect" during which time they may contribute risk of disease, but be neutral or even protective at other times (Murray et al. 1999). This appears to be particularly true in the pauciarticular groups, in which HLA-related risks clearly differ with age. For example, 50 % of children carrying at least one of the susceptibility MHC alleles have disease onset prior to their third birthday, suggesting that the period of susceptibility to early-onset pauciarticular JIA is limited to the first years of life. In contrast, HLA-B27 and DR-4 appear to be associated with the protection against early-onset pauciarticular JIA early in life, but with the increased risk for other forms of JIA later in childhood.

Predisposing Non-MHC Genes

The spectrum of non-HLA genes that may be associated with JIA is expanding rapidly. However, their contribution to the risk of JIA appears to be less significant than that of HLA genes, and their effects may not be specific to JIA. In general, the odds ratios are low, and distinguishing between founder effects (the incomplete mixing of genetically disparate populations) and associations that relate to pathogenesis has been difficult. Reproducible associations have been noted with interleukin-6, interleukin-10, and macrophage migration inhibitory factor (MIF) promoter polymorphisms (Fishman et al. 1998; Ogilvie et al. 2003; Crawley et al. 1999; Donn et al. 2001, 2002).

Several published genome-wide association studies (GWAS) in oligo- and polyarticular JIA produced compelling evidence for association with several loci also implicated in the genetics of other autoimmune diseases including adult rheumatoid arthritis. Examples include *PTPN2*, *PTPN22*,*IL2RA*, *ADAD1-IL2-IL21*, *ANGPT1*, *COG6*, *C12orf30*, and *STAT4* (Hinks et al. 2005, 2012; Thompson et al. 2004, 2012). Most of the loci have clear roles in the immune system. PTPN22 is an intracellular phosphatase that modulates cytokine signal transduction through JAK/STAT signaling pathways. PTPN2 is similar to PTPN22 and serves as a negative regulator of T cell activation. STAT4 is a transcriptional factor that mediates immune cell responses to Th1 cytokines. COG6 is a component of the conserved oligomeric Golgi complex involved in processes such as protein sorting and glycosylation. The *COG6* region has been recently associated with psoriasis. Angiopoietin-1 (ANGPT1) activates the receptor tyrosine kinase (TIE2), leading to increased expression of metalloproteinases as well as differentiation of smooth muscle and endothelial cells, generally leading to increased angiogenesis, a process that is important in the expansion of inflamed synovium in arthritis. Another recent GWAS study in oligo- and polyarticular JIA produced strong evidence for association with the chromosome 3q13 region, which includes *CD80*, a costimulatory molecule necessary for T cell activation (Hinks et al. 2012).

In summary, increasing evidence supports the concept that the occurrence of JIA and its phenotype is determined by complex genetic traits with "genetic interplay" between HLA and non-HLA predisposing alleles.

References

Clemens LE, Albert E, Ansell BM. Sibling pairs affected by chronic arthritis of childhood: evidence for a genetic predisposition. J Rheumatol. 1985;12:108.

Crawley E, Kay R, Sillibourne J, et al. Polymorphic haplotypes of the interleukin-10 5′ flanking region

determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum. 1999;42:1101.

Donn RP, Shelley E, Ollier WE, et al. A novel 5′-flanking region polymorphism of macrophage migration inhibitory factor is associated with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2001;44:1782.

Donn R, Alourfi Z, De Benedetti F, et al. Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. Arthritis Rheum. 2002;46:2402.

Fernandez-Viña MA, Fink CW, Stastny P. HLA antigens in juvenile arthritis. Pauciarticular and polyarticular juvenile arthritis are immunogenetically distinct. Arthritis Rheum. 1990;33:1787.

Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest. 1998;102:1369.

Førre O, Dobloug JH, Høyeraal HM, Thorsby E. HLA antigens in juvenile arthritis. Genetic basis for the different subtypes. Arthritis Rheum. 1983;26:35.

Glass D, Litvin D, Wallace K, et al. Early-onset pauciarticular juvenile rheumatoid arthritis associated with human leukocyte antigen-DRw5, iritis, and antinuclear antibody. J Clin Invest. 1980;66:426–9.

Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. 1987;30:1205.

Hall PJ, Burman SJ, Laurent MR, et al. Genetic susceptibility to early onset pauciarticular juvenile chronic arthritis: a study of HLA and complement markers in 158 British patients. Ann Rheum Dis. 1986;45:464.

Hinks A, Barton A, John S, et al. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. Arthritis Rheum. 2005;52:1694.

Hinks A, Cobb J, Sudman M, et al. Investigation of rheumatoid arthritis susceptibility loci in juvenile idiopathic arthritis confirms high degree of overlap. Ann Rheum Dis. 2012;71:1117.

Hoffman RW, Shaw S, Francis LC, et al. HLA-DP antigens in patients with pauciarticular juvenile rheumatoid arthritis. Arthritis Rheum. 1986;29:1057.

Melin-Aldana H, Giannini EH, Taylor J, et al. Human leukocyte antigen-DRB1*1104 in the chronic iridocyclitis of pauciarticular juvenile rheumatoid arthritis. J Pediatr. 1992;121:56.

Moroldo MB, Tague BL, Shear ES, et al. Juvenile rheumatoid arthritis in affected sibpairs. Arthritis Rheum. 1997;40:1962.

Moroldo MB, Chaudhari M, Shear E, et al. Juvenile rheumatoid arthritis affected sibpairs: extent of clinical phenotype concordance. Arthritis Rheum. 2004;50:1928.

Murray KJ, Moroldo MB, Donnelly P, et al. Age-specific effects of juvenile rheumatoid arthritis-associated HLA alleles. Arthritis Rheum. 1999;42:1843.0.

Nepom BS, Glass DN. Juvenile rheumatoid arthritis and HLA: report of the Park City III workshop. J Rheumatol Suppl. 1992;33:70.

Nepom BS, Nepom GT, Mickelson E, et al. Specific HLA-DR4-associated histocompatibility molecules characterize patients with seropositive juvenile rheumatoid arthritis. J Clin Invest. 1984;74:287.

Odum N, Morling N, Friis J, et al. Increased frequency of HLA-DPw2 in pauciarticular onset juvenile chronic arthritis. Tissue Antigens. 1986;28:245.

Oen K, Petty RE, Schroeder ML. An association between HLA-A2 and juvenile rheumatoid arthritis in girls. J Rheumatol. 1982;9:916.

Ogilvie EM, Fife MS, Thompson SD, et al. The −174G allele of the interleukin-6 gene confers susceptibility to systemic arthritis in children: a multicenter study using simplex and multiplex juvenile idiopathic arthritis families. Arthritis Rheum. 2003;48:3202.

Ombrello MJ, Remmers E, Grom AA, Thomson W, Martini A, Gattorno M, Ozen S, Bohnsack JF, Prahalad S, Zeft AS, Mellins ED, Satorius C, Langefeld CD, Zeggini E, Glass DN, Thompson SD, Kastner DL, Woo P. International Childhood Arthritis Genetics Consortium. Major histocompatibility class II gene cluster harbors systemic juvenile idiopathic arthritis susceptibility locus. Arthritis Rheum. 2011; 63 Suppl 10:1686.

Petty RE, Southwood TR, Baum J, Bhettay E, Glass DN, Manners P, et al. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. J Rheumatol. 1998;25:1991–5.

Ploski R, Vinje O, Rønningen KS, Spurkland A, Sørskaar D, Vartdal F, Førre O. HLA class II alleles and heterogeneity of juvenile rheumatoid arthritis. DRB1*0101 may define a novel subset of the disease. Arthritis Rheum. 1993;36:465–72.

Prahalad S, Shear ES, Thompson SD, Giannini EH, Glass DN. Increased prevalence of familial autoimmunity in simplex and multiplex families with juvenile rheumatoid arthritis. Arthritis Rheum. 2002;46:1851.

Rachelefsky GS, Terasaki PI, Katz R, Stiehm ER. Increased prevalence of W27 in juvenile rheumatoid arthritis. N Engl J Med. 1974;290:892.

Reekers P, Schretlen ED, van de Putte LB. Increase of HLA-"DRw6" in patients with juvenile chronic arthritis. Tissue Antigens. 1983;22:283.

Rossen RD, Brewer EJ, Sharp RM, et al. Familial rheumatoid arthritis: linkage of HLA to disease susceptibility locus in four families where proband presented with juvenile rheumatoid arthritis. J Clin Invest. 1980;65:629.

Schaller JG, Ochs HD, Thomas ED, et al. Histocompatibility antigens in childhood-onset arthritis. J Pediatr. 1976;88:926.

Stastny P, Fink CW. Different HLA-D associations in adult and juvenile rheumatoid arthritis. J Clin Invest. 1979;63:124.
Thompson SD, Moroldo MB, Guyer L, et al. A genome-wide scan for juvenile rheumatoid arthritis in affected sibpair families provides evidence of linkage. Arthritis Rheum. 2004;50:2920.
Thompson SD, Marion MC, Sudman M, et al. Genome-wide association analysis of juvenile idiopathic arthritis identifies a new susceptibility locus at chromosomal region 3q13. Arthritis Rheum. 2012;64:2781.
Towner SR, Michet Jr CJ, O'Fallon WM, Nelson AM. The epidemiology of juvenile arthritis in Rochester, Minnesota 1960–1979. Arthritis Rheum. 1983;26:1208.
Van Kerckhove C, Melin-Aldana H, Elma MS, et al. A distinct HLA-DRw8 haplotype characterizes patients with juvenile rheumatoid arthritis. Immunogenetics. 1990;32:304.
van Kerckhove C, Luyrink L, Taylor J, et al. HLA-DQA1*0101 haplotypes and disease outcome in early onset pauciarticular juvenile rheumatoid arthritis. J Rheumatol. 1991;18:874.

Gestational Alloimmune Liver Disease

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Synonyms

Neonatal hemochromatosis

Definition

Gestational alloimmune liver disease (GALD) is a unique fetal liver disease that results from maternal alloimmunity against fetal hepatocytes. Neonatal hemochromatosis (NH) is a clinical syndrome in which liver disease in the newborn is accompanied by siderosis of extrahepatic tissues in the pattern seen in hereditary hemochromatosis. NH is the phenotypic expression of severe fetal liver disease. GALD is the cause of fetal liver injury leading to nearly all instances of NH. However, GALD may also produce acute liver failure in the fetus and severe acute neonatal liver disease often with no extrahepatic siderosis.

Etiology and Pathogenesis

GALD is mediated by immunoglobulin G (IgG) as are all maternofetal alloimmune diseases (Hoftman et al. 2008). Maternal antibodies of the IgG class are actively transported across the placenta to the fetus from about the 12th week of gestation when the neonatal crystallizable fragment receptor (FcRn, the IgG chaperone) is first expressed. The principal function of this process is to provide humoral immunity for the fetus and newborn. The principle of gestational alloimmunity involves exposure of the mother to a fetal antigen that she fails to recognize as "self," which results in sensitization and production of specific reactive IgG. GALD-related alloimmunity is specifically directed at fetal hepatocyte-specific antigen. No non-hepatocyte elements in the liver appear to be injured, and tissues outside the liver are unaffected by the primary immune process. Once sensitization has occurred maternal, anti-fetal liver IgG is passed to the fetus where it binds to the liver antigen and results in immune injury of the fetal liver. The mechanism of hepatocyte injury appears to involve the fetal innate immune system. The terminal complement cascade is activated by the classical pathway and results in the formation of membrane attack complex (Pan et al. 2010). Immunohistochemical staining for complement complex C5b-9 (the neoantigen created during terminal complement cascade activation and culmination) shows nearly all hepatocytes in cases of GALD to have complement-mediated injury (Pan et al. 2010). This has become the defining feature of GALD.

Liver Pathology

GALD is defined by complement-mediated hepatocyte injury and is the only known liver disease to have this as the primary disease mechanism

(Pan et al. 2010; Whitington et al. 2011). The anatomic pathology of the liver often resembles that seen in acute and subacute liver failure in older individuals. Marked loss of hepatocyte mass is a prominent feature of most cases, and in some instances, almost no hepatocytes remain. The residual and/or regenerating hepatocytes may exhibit either giant-cell or pseudoacinar transformation with canalicular bile plugs. Fibrosis is pronounced, particularly in the lobule and around the central vein. Portal triads are spared from injury and fibrosis. Regenerative nodules may be present. Residual hepatocytes often show siderosis while Kupffer cells are spared. Inflammation is minimal. Parenchymal inflammation consists of macrophages and some neutrophils. These are the inflammatory elements of the innate immune system that are recruited by activation of the terminal complement cascade via production of complement component fragments C3a and C5a.

GALD may produce acute injury to the fetal liver and has been associated with fetal death and stillbirth (Whitington et al. 2011). The pathology in such cases shows acute hepatocyte necrosis with no collapse, no fibrosis, and no inflammation, indicating the hyperacuity of the process. Many of these cases have no siderosis of the liver or other tissues. In addition, several term newborns with acute liver failure have been found to have GALD with pathology similar to that in cases of fetal death due to GALD. It is not at all clear why some cases present with acute liver failure while others present with congenital cirrhosis.

Extrahepatic Manifestations of GALD

Extrahepatic Siderosis

Siderosis of tissues outside the liver is the defining feature of the NH phenotype (Whitington 2006; Whitington and Fleming 2008). The most consistently affected tissues are the acinar epithelium of the exocrine pancreas, myocardium, the epithelia of the thyroid follicles, and the oral mucosal ("minor salivary") glands. The spleen, lymph nodes, and bone marrow contain comparatively trivial quantities of stainable iron.

The fetal liver controls the flow of iron from the mother to the fetus just as intestinal iron absorption is controlled after birth, by sensing iron sufficiency and producing hepcidin as a regulatory feedback molecule. The efflux of iron from the placenta is dependent upon ferroportin, just as it is in the intestine and reticuloendothelial system. Hepcidin regulates ferroportin functional expression by binding to it and causing it to undergo internalization and proteosomal degradation. Liver from fetal and newborn GALD patients expresses hepcidin at a small fraction of that expressed by normal fetal and newborn liver (Bonilla et al. 2012). The liver injury-induced failure to produce adequate hepcidin to appropriately regulate placental iron flux fully explains iron overload in GALD and probably also in other fetal liver diseases leading to the NH phenotype (Zoller and Knisely 2012).

The specific distribution of iron in extrahepatic tissues in the NH phenotype requires additional explanation. These same tissues do not show siderosis in normal newborns whose plasma contains transferrin-bound iron. Thus, development of siderosis appears to be a function of excess non-transferrin-bound iron (NTBI). Examination of normal newborns for expression of uptake carriers for NTBI showed that all tissues affected by siderosis in GALD-NH expressed solute carrier family 39 (zinc transporter), member 14 (ZIP14), a known NTBI transporter (Bonilla et al. 2012). However, several tissues that expressed ZIP14 are not subject to siderosis in GALD-NH. These tissues also expressed ferroportin for export of iron. Thus, in normal newborn tissues, those expressing ZIP14 and not ferroportin are uniquely susceptible to siderosis in conditions of excess NTBI. Tissues from GALD-NH cases showed the same pattern of carrier expression and siderosis in the ZIP14-positive, ferroportin-negative tissues. It should be remembered that ferroportin is fully expressed in GALD-NH despite iron overload because of the liver's incapacity to make hepcidin. This is why reticuloendothelial cells show no siderosis in NH despite iron overload.

Angiotensinogen Deficiency-Related Renal Dysgenesis

Occasionally severely affected babies exhibit renal hypoplasia, with dysgenesis of proximal tubules and paucity of peripheral glomeruli. Correlation with the process of normal renal development dates this arrest of renal development to about 24 weeks gestation. This final stage of renal development is dependent upon liver function, specifically the synthesis of angiotensinogen. The livers and kidneys of infants with GALD-NH were studied in comparison to normal newborns dying of perinatal asphyxia to examine this process (Bonilla et al. 2010). Liver angiotensinogen expression correlated closely with the mass of hepatocytes as determined by quantitative morphometry. Hepatocyte mass and angiotensinogen gene expression were markedly reduced in GALD-NH relative to normal. Proximal tubule development was studied using quantitative morphometry of kidney sections stained by immunohistochemistry for the specific marker fumaryl acetoacetic acid hydrolase (FAH). All GALD-NH cases had a degree of dysgenesis of proximal tubules, which is a surprising finding given so few cases had been reported. Furthermore, the density of proximal tubules correlated closely with the hepatic angiotensinogen gene expression. In sum, it appears that in GALD alloimmune injury leads to reduced hepatocyte mass, which results in reduced angiotensinogen production, which in turn leads to defective renal development. Therefore, the disordered development dates fetal liver failure to the late second and early third trimester.

Clinical Findings and Diagnosis

The predominant presenting findings are those of liver failure and often multiorgan failure (Whitington and Fleming 2008). Hypoglycemia, marked coagulopathy, hypoalbuminemia and edema with or without ascites, and oliguria are prominent features. Jaundice develops during the first few days after birth. Most cases exhibit significant elevations of both conjugated and nonconjugated bilirubin, with total bilirubin levels often exceeding 30 mg/dl. Serum aminotransferase concentrations are disproportionately low for the degree of hepatic injury; indeed, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are typically normal or low and rarely exceed 100 IU/L. Serum α-fetoprotein (AFP) levels are characteristically very high, usually 100,000–600,000 ng/ml (normal term newborn values <80,000 ng/ml). Studies of iron status often show low transferrin levels and iron saturation nearly always exceeding 95 % (Bonilla et al. 2012). The low transferrin levels probably reflect severe liver disease. Elevated serum ferritin (values >800 ng/ml) is characteristic of GALD-NH but is a nonspecific finding in neonatal liver disease.

The time of presentation extends from 18-weeks gestation to term plus 2–3 months and the mode from acute liver failure to subclinical liver disease. One of GALD's most under recognized presentations is late-second and third-trimester fetal loss. The gestational histories of women who have had a baby diagnosed with NH show that approximately one in seven pregnancies end with fetal loss. In an autopsy study, severe acute GALD has been associated with fetal death and stillbirth, presumably by causing acute liver failure in the fetus (Whitington et al. 2011). Most affected live-born babies show evidence of fetal insult (i.e., intrauterine growth restriction and oligohydramnios) and premature birth is common. Liver disease is generally apparent within hours of birth, and NH is one of the most common causes of liver failure in the neonate (Durand et al. 2001; Rodrigues et al. 2005). In occasional cases the liver disease takes a prolonged course and is manifest days to weeks after birth. There is a spectrum of disease, and some infants recover with supportive care. Indeed, it may be that some "affected" babies have no clinical disease. Twins may have disparate clinical findings, with one severely affected and the other minimally so (Ekong et al. 2005).

The approach to diagnosing GALD-NH relies mainly on diagnosing NH, since nearly all NH is due to GALD. It should be suspected in infants who manifest liver disease before or very shortly after birth. It should also be suspected in unexplained cases of stillbirth and newborn death. Demonstration of extrahepatic siderosis is

necessary to make the diagnosis of NH. Finding siderosis in the liver is not diagnostic. The normal newborn liver contains sufficient stainable iron to be confused with pathologic siderosis, although they are qualitatively different to the eyes of experienced pathologists. Furthermore, pathologic hepatic siderosis has been described in several neonatal liver diseases, usually not in combination with extrahepatic siderosis. The absence of stainable iron in the liver does not exclude the diagnosis since in many cases few if any hepatocytes remain, and hepatic siderosis in NH involves hepatocytes exclusively.

NH is often diagnosed at autopsy, where siderosis of many tissues can be demonstrated if looked for. Proper stains for iron should be performed on the tissues typically involved when autopsy is performed on any baby with liver failure or suspected liver disease and in unexplained stillbirths and newborn deaths. Demonstration of extrahepatic siderosis in living babies can be by tissue biopsy or by magnetic resonance imaging (MRI). Biopsy of the oral mucosa is a clinically useful approach to obtain glandular tissue in which to demonstrate siderosis (Knisely et al. 1988). Differences in magnetic susceptibility between iron-laden and normal tissues on T2-weighted MRI can document siderosis of various tissues, particularly the pancreas and liver (Udell et al. 2005). The diagnostic utility of these approaches has never been formally evaluated. Oral mucosal biopsy often fails because an inadequate specimen is obtained, one not containing submucosal glands. Experience suggests that adequate biopsies contain some stainable iron (any amount is abnormal) in about two-thirds of cases with autopsy diagnosis of NH, whereas MRI demonstrates abnormal iron distribution also in about two-thirds of such cases. The recommended diagnostic approach is to perform one (depending on ease and availability) and only if that is negative, do the other. There is no need to do both simultaneously. GALD can be diagnosed in the absence of NH, which is to say in the absence of extrahepatic siderosis, using immunohistochemistry for C5b-9 in liver biopsy (Whitington et al. 2011; Debray et al. 2012).

The following diagnostic approach should minimize missed diagnoses. In a newborn with liver failure or other suspicious clinical circumstances (e.g., nonimmune hydrops), an attempt should be made to demonstrate extrahepatic siderosis by oral mucosal biopsy and/or MRI. If positive, the diagnosis of NH is established. One can consider sending appropriate tests to rule out the non-GALD causes of NH at this point (i.e., bile acid synthesis defect and mitochondrial DNA depletion due to deoxyguanosine kinase (DGUOK) mutations). If negative, liver biopsy for C5b-9 staining can be considered. However, any newborn with liver failure and no identifiable other causes (e.g., perinatal herpes) should be considered to have GALD and treated accordingly (see below). Autopsy diagnosis is likewise straightforward. Stillbirths undergoing post-mortem should be examined for extrahepatic siderosis unless there is a clear cause for fetal death identified. Stillborn liver is often macerated, so a diagnosis of acute liver injury is obscured. C5b-9 staining is difficult to perform and interpret in macerated liver, so it cannot be recommended in such cases. Newborns dying of liver failure or under other suspicious circumstances and undergoing post-mortem examination should be examined for extrahepatic siderosis. Liver disease can usually be diagnosed by histopathology in these circumstances; however, the changes of acute GALD may be ascribed to post-mortem changes. Thus, in cases suspicious for GALD but with no extrahepatic siderosis, C5b-9 staining should be considered.

Prevention and Treatment

GALD shows an unusual pattern and high rate of recurrence in the progeny of affected women. After the index case there is an approximately 90 % probability that each subsequent baby born to that mother will be affected (Whitington and Kelly 2008). However, severe NH can be prevented by treatment during gestation (Whitington and Kelly 2008). The current guideline for treatment consists of intravenous immunoglobulin administered at a dose of 1 g/kg body weight (high-dose IVIG) at 14 weeks, 16 weeks, and weekly from the 18th week until the end of

gestation. Women whose most recent gestation was affected with proven NH should be treated in lieu of any other markers for high risk of recurrence. This treatment in over 100 pregnancies has resulted in >98 % success as defined by pregnancy outcome of children who are currently healthy and with no liver disease. Treatment with high-dose IVIG during gestation effectively modifies recurrent GALD so that it is not lethal to the fetus or the newborn.

Medical therapy of infants with severe GALD-NH using the combination of double-volume exchange transfusion to remove existing reactive antibody and administration of high-dose IVIG (1 g/kg) to block antibody action (i.e., interfere with complement activation) improves survival as compared to historical controls (Rand et al. 2009). Over 50 infants have been treated in this manner with approximately 80 % survival with medical therapy alone. These results may be biased by non-report of poor outcomes or treatment of infants not having NH or only mild disease, since infants have been treated around the world not on protocol and the tally relies on often spontaneous reporting by email. Yet, this appears to be an important advance in treating NH, and it can be applied in less than advanced medical environments. The recommended approach to treating NH is as follows. When presented with an infant in liver failure, if one even thinks it could be NH, a dose of IVIG should be given at once. Administration of IVIG is associated with few complications, and a single dose poses little or no risk to a newborn, no matter the disease. Then a formal evaluation can begin. If NH is proven and the infant has not shown significant clinical improvement, then exchange transfusion can be performed followed by a second dose of IVIG. Improvement in liver function may be delayed because this treatment can only reduce ongoing immune-mediated injury. These severely injured livers require regeneration and/or hepatocyte recovery before function improves. Normalization of the INR takes on average 4–6 weeks.

NH is a frequent indication for liver transplantation in the first 3 months of life (Rodrigues et al. 2005; Grabhorn et al. 2006). When performed for NH, the difficulties attendant to transplanting newborns are frequently compounded by prematurity, small size for gestational age, and multiorgan failure. The overall survival of infants receiving a liver transplant for the indication of NH is about 35 %. Furthermore, it is clear that babies with clinical liver failure due to NH can fully recover with medical therapy. So, caution is indicated when considering treating an affected baby with liver transplantation.

References

Bonilla SF, Melin-Aldana H, et al. Relationship of proximal renal tubular dysgenesis and fetal liver injury in neonatal hemochromatosis. Pediatr Res. 2010;67:188–93.

Bonilla S, Prozialeck JD, et al. Neonatal iron overload and tissue siderosis due to gestational alloimmune liver disease. J Hepatol. 2012;56:1351–5.

Debray FG, de Halleux V, et al. Neonatal liver cirrhosis without iron overload caused by gestational alloimmune liver disease. Pediatrics. 2012;129:e1076–9.

Durand P, Debray D, et al. Acute liver failure in infancy: a 14-year experience of a pediatric liver transplantation center. J Pediatr. 2001;139:871–6.

Ekong UD, Kelly S, et al. Disparate clinical presentation of neonatal hemochromatosis in twins. Pediatrics. 2005;116:e880–4.

Grabhorn E, Richter A, et al. Neonatal hemochromatosis: long-term experience with favorable outcome. Pediatrics. 2006;118:2060–5.

Hoftman AC, Hernandez MI, et al. Newborn illnesses caused by transplacental antibodies. Adv Pediatr. 2008;55:271–304.

Knisely AS, O'Shea PA, et al. Oropharyngeal and upper respiratory tract mucosal-gland siderosis in neonatal hemochromatosis: an approach to biopsy diagnosis. J Pediatr. 1988;113:871–4.

Pan X, Kelly S, et al. Novel mechanism of fetal hepatocyte injury in congenital alloimmune hepatitis involves the terminal complement cascade. Hepatology. 2010;51:2061–8.

Rand EB, Karpen SJ, et al. Treatment of neonatal hemochromatosis with exchange transfusion and intravenous immunoglobulin. J Pediatr. 2009;155:566–71.

Rodrigues F, Kallas M, et al. Neonatal hemochromatosis – medical treatment vs. transplantation: the king's experience. Liver Transpl. 2005;11:1417–24.

Udell IW, Barshes NR, et al. Neonatal hemochromatosis: radiographical and histological signs. Liver Transpl. 2005;11:998–1000.

Whitington PF. Fetal and infantile hemochromatosis. Hepatology. 2006;43:654–60.

Whitington PF, Fleming RE. Hemochromatosis. In: Kleinman RE, Goulet O-J, Sherman PM, Sanderson IR, Shneider BL, Walker WA, editors. Walker's pediatric gastrointestinal disease: pathophysiology, diagnosis management. Hamilton: BC Decker; 2008. p. 1049–56.
Whitington PF, Kelly S. Outcome of pregnancies at risk for neonatal hemochromatosis is improved by treatment with high-dose intravenous immunoglobulin. Pediatrics. 2008;121:e1615–21.
Whitington PF, Pan X, et al. Gestational alloimmune liver disease in cases of fetal death. J Pediatr. 2011;159:612–6.
Zoller H, Knisely AS. Control of iron metabolism – lessons from neonatal hemochromatosis. J Hepatol. 2012;56:1226–9.

Giant Cell Arteritis

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Synonyms

Giant cell arteritis: Temporal arteritis; Horton's disease

Definition

Giant cell arteritis (GCA): Chronic vasculitis of large and medium-sized arteries with a specific predilection for aorta and its proximal branches in individuals after the age of 50.

Definition – Criteria

Giant cell arteritis (GCA) is an idiopathic arteritis that affects predominantly large- and medium-sized arteries containing an elastic lamina layer, exclusively in individuals after the age of 50 (Salvarani et al. 2012; Borchers and Gershwin 2012; Weyand and Goronzy 2012). The most commonly affected arteries are the aorta and its proximal branches. It can involve the superficial temporal artery and occasionally the ophthalmic and posterior ciliary arteries leading to its most dreadful complication, i.e., blindness.

The American College of Rheumatology (ACR) has developed specific classification criteria for the disease in 1990 (Hunder et al. 1990). A patient should fulfill at least three of five proposed criteria in order to be classified as having GCA (age >50 years, new onset headache, tenderness or decreased palpation of temporal artery, elevated erythrocyte sedimentation rate-ESR >50 mm/h, abnormal artery biopsy).

Epidemiology

GCA is the most common form of systemic vasculitis after the age of 50; its incidence ranges as low as 1 new case/100,000/year in Turkey or African and Asian countries to 30 cases/100,000/year in Scandinavian countries (Borchers and Gershwin 2012; Weyand and Goronzy 2012). Women are affected more commonly than men (2:1).

Pathogenesis

GCA is a typical inflammatory arteritis with inflammation that involves all layers of the arterial wall, frequently leading to vascular stenosis and/or occlusion (Fig. 1) (Weyand and Goronzy 2012). The main population of infiltrating inflammatory cells consists of CD4+ T cells, macrophages, and multinucleated giant cells (which is why the disease is called giant cell arteritis) (Weyand and Goronzy 2012). These histologic features and extensive animal and human data have shown that a predominant Th1 (increased expression of interferon-γ/IFN-γ and interleukin-12/IL-12 and IL-32) and Th17 (increased expression of IL-17) response is present in the arterial wall. These cytokines together with other proinflammatory or antiangiogenic cytokines, such as IL-6, IL-1β, tumor necrosis factor, platelet-derived growth factor, free oxygen radicals, and metalloproteinases (MMP-2 and MMP-9), are responsible

for the systemic inflammatory symptoms and findings (fever, fatigue, weight loss, increased ESR and C-reactive protein – CRP), and the characteristic changes seen in the arterial wall (e.g., disruption of the internal elastic lamina, vascular occlusion).

Clinical Manifestations

The clinical manifestations of GCA depend on the site(s) of vascular involvement (Weyand and Goronzy 2012). Two types of involvement have been recognized: the cranial and the large vessel type.

The **cranial type** is the most common and is manifested by inflammation of the branches of the external carotid artery. Patients usually present with daily temporal headache (75 %) unresponsive to analgesics accompanied by scalp tenderness and jaw claudication. Involvement of the posterior ciliary or retinal arteries can lead to damage to the optic nerve and visual manifestations such as diplopia and transient or permanent loss of vision. Blindness occurs in approximately 15 % of patients with GCA during the early stages of the disease.

Large vessel arteritis involving the aorta (thoracic more than abdominal) and its branches (subclavian, axillary, lower extremity arteries) is increasingly recognized as a common manifestation of GCA (Salvarani et al. 2012). Using newer imaging techniques such as computed tomography angiography (CTA) or ^{18}F-FDG-positron emission tomography (PET) scan, subclinical involvement of large arteries is present at diagnosis in 30–70 % of cases. Clinical manifestations resulting from the formation of vascular stenoses or aneurysms are less frequent, occurring in less than 15 % of patients during the course of the disease (Weyand and Goronzy 2012; Salvarani et al. 2012).

Regardless of the site(s) of involvement (cranial and/or large vessel), almost half of the patients display systemic manifestations such as fever, weight loss, fatigue or aching, and stiffness in shoulders and hips (polymyalgia rheumatica or PMR). Fever without signs or symptoms of vascular involvement can be the only presenting symptom in a small percentage of patients with a fever of unknown origin (FUO) (Salvarani et al. 2012).

Diagnostic Assesments

Laboratory Tests

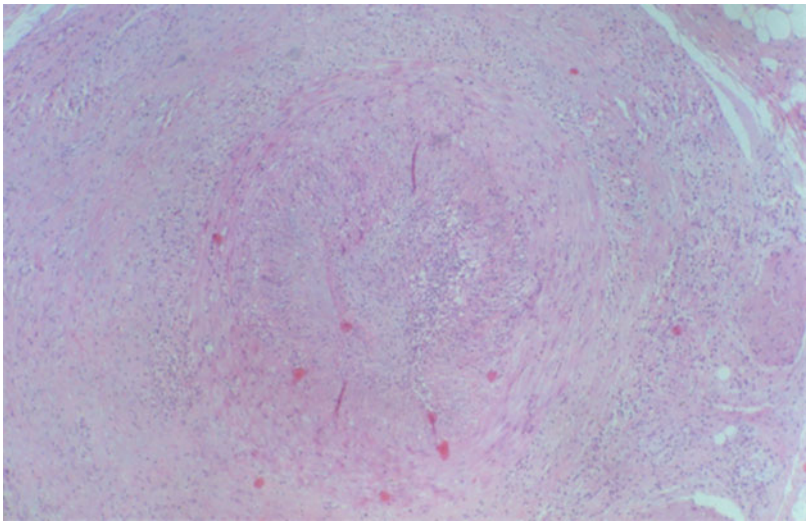
Simple markers of inflammation such as the ESR and the CRP are elevated in the majority of patients with GCA (~95 %) (Salvarani et al. 2012). Thus, a normal ESR and CRP remain the best initial tests to rule out this disease. On the other hand, it should be emphasized that in patients with typical clinical picture, a normal ESR and CRP should not exclude this diagnosis, until further workup has been performed, especially if these tests were performed while the patient was taking corticosteroids.

Temporal Artery Biopsy

Biopsy of an involved superficial temporal artery branch remains the best way to definitely establish the diagnosis of GCA (Salvarani et al. 2012; Weyand and Goronzy 2012). When an adequate size specimen is sampled (>2 cm), the biopsy is positive in 80–90 % of cranial and 40–50 % of large vessel GCA. Typical findings include a panarteritis involving the adventitia, media, and intimal layers of the artery (see Fig. 1); giant cells are present in 50 % of the cases around the elastic lamina (intima-media junction). There is currently no consensus regarding whether the initial biopsy should be unilateral or bilateral.

Imaging

A number of imaging modalities have been developed that are helpful in the diagnosis of cranial and more importantly large vessel GCA (Salvarani et al. 2012; Weyand and Goronzy 2012). These include color Doppler ultrasonography (US), magnetic resonance angiography (MRA), CTA, and ^{18}F-FDG-PET scan (Salvarani et al. 2012). US of the superficial temporal arteries has a sensitivity of 68–75 % and a specificity of 83–91 % for GCA diagnosis and could be



Giant Cell Arteritis, Fig. 1 Temporal artery biopsy of a patient with giant cell arteritis showing a panarteritis leading to occlusion of the vessel lumen

a useful tool in experienced hands to identify an abnormal artery for biopsy. MRA, CTA, and PET scan are needed for the diagnosis of large vessel GCA (aorta involvement), especially during the early stages of the disease. Their exact role in the follow-up of these patients has not been established.

Differential Diagnosis

For patients over the age of 50 presenting with the classical clinical features of cranial GCA, the diagnosis is not difficult to make. Rarely, small or medium size vasculitides or noninflammatory vasculopathies that involve the vessels surrounding the temporal artery can mimic GCA. These include ANCA-associated vasculitides (Granulomatosis with polyangiitis (Wegener's), microscopic polyangiitis (MPA), Churg-Strauss syndrome), polyarteritis nodosa, amyloidosis, or thromboangiitis obliterans (Salvarani et al. 2012). The typical histological features of the disease can easily differentiate these entities.

Large vessel vasculitis or GCA presenting only as FUO can create diagnostic difficulties since the differential diagnosis is wide and includes a number of infectious, neoplastic, and inflammatory disorders that present with nonspecific systemic manifestations (Salvarani et al. 2012). Temporal artery biopsy and appropriate imaging of aorta and its branches can aid in the correct diagnosis after other diseases have been ruled out with appropriate workup.

Treatment

The treatment of GCA (cranial or large vessel) should be started as early as possible in order to diminish the risk of blindness (Salvarani et al. 2012). This is the case even for patients without a positive temporal artery biopsy, if the clinical suspicion is sufficiently high. Despite the significant advances that have been made in the treatment of rheumatic disorders, corticosteroids remain the cornerstone of treatment. Patients are usually started with oral prednisone (40–60 mg/day) with gradual tapering of the dose that should reach 10–15 mg/day after 3 months (Mukhtyar et al. 2009; Dasgupta et al. 2010). Patients with threatened or established visual loss usually receive higher doses initially (either intravenously as pulses or by mouth). Although the majority of patients are able to discontinue steroids after approximately 2 years, relapses are frequent (40–50 %) during therapy requiring increase in the dose of steroids (Salvarani et al. 2012). Long-term therapy and high accumulating dose of steroids are associated with a number of significant side effects such as osteoporotic fractures, cataracts, infections,

hypertension, and diabetes mellitus (Salvarani et al. 2012; Weyand and Goronzy 2012).

In addition to corticosteroids, low-dose aspirin is recommended (unless contraindicated) as it may reduce the incidence of vision loss (Nesher et al. 2004).

Prophylactic anti-osteoporotic treatment is recommended for all patients treated for GCA, while there have been a number of studies assessing the role of steroid sparing agents for the treatment of GCA (Mukhtyar et al. 2009; Dasgupta et al. 2010). Among them the best studied is methotrexate with conflicting results regarding its efficacy. Newer biologic agents such as the IL-6 antagonist (tocilizumab) have shown promising results but controlled trials proving their usefulness in GCA are needed (Salvarani et al. 2012).

Cross-References

- ▶ Polymyalgia Rheumatica
- ▶ Vasculitis: Granulomatosis with Polyangiitis (Wegener's)

References

Borchers AT, Gershwin ME. Giant cell arteritis: a review of classification, pathophysiology, geoepidemiology and treatment. Autoimmun Rev. 2012;11:A544–54.

Dasgupta B, Borg FA, Hassan N, Alexander L, Barraclough K, Bourke B, Fulcher J, Hollywood J, Hutchings A, James P, Kyle V, Nott J, Power M, Samanta A. BSR and BHPR guidelines for the management of giant cell arteritis. Rheumatology (Oxford). 2010;49:1594–7.

Hunder GG, Bloch DA, Michel BA, Stevens MB, Arend WP, Calabrese LH, Edworthy SM, Fauci AS, Leavitt RY, Lie JT. The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. Arthritis Rheum. 1990;33:1122–8.

Mukhtyar C, Guillevin L, Cid MC, Dasgupta B, de Groot K, Gross W, Hauser T, Hellmich B, Jayne D, Kallenberg CG, Merkel PA, Raspe H, Salvarani C, Scott DG, Stegeman C, Watts R, Westman K, Witter J, Yazici H, Luqmani R. EULAR recommendations for the management of large vessel vasculitis. Ann Rheum Dis. 2009;68:318–23.

Nesher G, Berkun Y, Mates M, et al. Low-dose aspirin and prevention of cranial ischemic complications in giant cell arteritis. Arthritis Rheum. 2004;50(4):1332.

Salvarani C, Pipitone N, Versari A, Hunder GG. Clinical features of polymyalgia rheumatica and giant cell arteritis. Nat Rev Rheumatol. 2012;8:509–21.

Weyand CM, Goronzy JJ. Giant cell arteritis. In: Hoffman GS, Weyand CM, Langford CA, Goronzy JJ, editors. Inflammatory diseases of blood vessels. Chichester: Wiley-Blackwell; 2012. p. 263–75.

Gut Microbiome, Overview

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Synonyms

Gut microbiome; Gut microbiota; Inflammatory bowel disease; Metagenome; Probiotics

Definition

"Gut microbiome" describes the combined suite of genes of all microbiota present in the gut. It enlarges the host's gene pool 150-fold and influences health and disease. In order to illustrate the importance of the microbiome, this entry will first give an overview of its origins, normal function, and composition and, then, focus on how microbial changes affect human health. Further definitions are listed in Table 1.

Normal Gut Microbiota

In the gut, human cells are outnumbered by a vast, complex, and dynamic consortium of microorganisms, the gut microbiota (Ley et al. 2006). A refined symbiosis provides a protected habitat with constant nutrient supply for the microbes. In return the microbiota offers plenty of support in nutrient degradation and uptake where human cells alone lack the appropriate enzymes (Qin et al. 2010). Other functions influenced by the microbiota include fat metabolism, epithelial homeostasis,

Gut Microbiome, Overview, Table 1 Definitions

Gut microbiota	All microorganisms of the gut microbial community (bacteria, archaea, protozoa, fungi, viruses)
Gut microbiome	All genes present in the gut microbial community
Gut metagenome	All human and all microbial genes in the human gut combined
Microbial community	All microorganisms sharing a habitat/niche, e.g., the human gut or the colonic or ileal mucosa
Core microbiome	Set of microbial genes which can be found in most individuals of a host species; the contained genes convey important functions for the host organism
Microflora	Outdated term for microbiota ("flora" suggesting plant origin)
Probiotics	Certain microbes said to have anti-inflammatory or immune regulatory properties

angiogenesis, enteric nerve function, pathogen resistance, development, and immune homeostasis (Hill and Artis 2010). Modern sequencing techniques have shifted the focus onto microbial genes and the microbiome as a whole rather than species. Functionally, the abundance of certain genes is more important than which bacterium provides them. Besides, bacteria are known to exchange genetic information via plasmids, short loops of DNA, which can be transferred to a different bacterial cell. The terms microbiota and microbiome are often used are often used interchangeably to describe the same thing though the focus is in fact slightly different.

Composition of the Microbiota

Our knowledge of the mammal microbiome is still fairly young. Only in 1991 Weisburg et al. reported on a novel 16S rRNA approach to identify non-culturable microbial species via phylogenetic description. The gain of knowledge since has been exponential, jumping from 300 culturable species to current estimates of 1,800 genera and 15,000–36,000 species newly identified by culture-independent techniques (Weisburg et al. 1991). And still, while the size of the known microbiome has exploded, large parts of its function have yet to be deplored (Frank et al. 2007).

An important focus has been on a common microbial core, which is thought to hold the most important genes. Early reports still saw high interindividual differences (Eckburg et al. 2005; Ley et al. 2006; Frank et al. 2007). However, a recent large multicenter study using advanced deep sequencing techniques has in fact reported an important overlap: 38 % of genes as well as 18–75 of microbial species were shared among individuals, thus describing a common microbial core on the gene and the phylogenetic level (Qin et al. 2010).

However, the gut microbial community is not uniform. The microbial composition of stool is different than that on the mucosal surface. Biofilms connect different species with each other as well as with the epithelium and its overlaying mucus layer. Luminal bacteria on the other hand are considered planctonic, free floating. These varying subcommunities convey different niches for bacteria and different functions for the host (Sonnenburg et al. 2004; Eckburg et al. 2005). Upon closer look, even the gastrointestinal mucosa itself harbors varying communities. Many colonic species specialize in the degradation of fibers whereas the small intestine harbors more bacteria geared towards sugar degradation (e.g., the small intestine harbors less Bacteroidetes and Lachnospiraceae but has a higher prevalence of Streptococcaceae, Actinomycaeae, and Corynebacteriaceae (Frank et al. 2007)). However, other authors have suggested that incomplete colonic peristalsis with backflow and mixing can keep the mucus-associated microbiota relatively homogenous. While these views are not necessarily contradictory, further technologic advances and an increase in cohort size will lead to a better understanding of special niches.

Development and Ontogeny of Microbial Communities

Before birth mammals are believed to be sterile. Early colonization occurs during the first days and weeks of life more or less by chance of first contact. According studies have shown high

temporal and interindividual variation during this period. Over time, however, these preliminary communities are replaced by other microbes which are more adapted to the human gut niche. Thus, at approximately 1 year of age, broad microbial contact and food variety have shaped a typically human, adult-like microbiota that remains more or less stable. External factors influencing this process are mode of delivery, hospitalization, use of antibiotics, country of birth, prematurity, and feeding practices. A higher similarity has also been observed among relatives of several generations, suggesting a vertical inheritance ("parent-to-offspring hypothesis") (Hill and Artis 2010).

Compared to the outside world, our gut is a very selective environment. High selection pressure exists both from the host's immune system ("top-down selection") and from competing microbes ("bottom-up selection"). Phylogenetic analysis shows that almost 90 % of all gut bacteria belong to the phyla of Firmicutes (56 %) and Bacteroidetes (23 %) which in the soil and the ocean make up less than 10 % (Eckburg et al. 2005). However, although the human gut harbors a high diversity of subspecies and strains, the actual species level is rather shallow with approximately 400–500 phyla. In evolutionary terms this suggests that the initial colonization of the gut reservoir was accomplished by only a limited population of microbes that then could diversify easily ("bottleneck population and détente") (Eckburg et al. 2005; Ley et al. 2006), a bit like Darwin's finches in the Galapagos archipelago.

Gut Microbiota and Inflammatory Bowel Disease

Evidence for Microbial Involvement

The idea of microbial involvement in inflammatory bowel disease (IBD) is not new. In their 1932 landmark paper "Regional Ileitis," Crohn, Ginzburg, and Oppenheimer presented a new pathologic and clinical entity and discussed whether it might be due to "localized decrease in resistance to some bacterial invasion" (Crohn et al. 1984). Eighty years later microbial involvement is a known fact, but the mechanisms are still unclear.

Evidence for microbial involvement comes from clinical and experimental observations. Use of antibiotics can attenuate acute flares. Parts of the bowel that have been excluded from the fecal stream through surgery stay inflammation free until reconnection and consecutive microbial reexposure. In experimental settings, genetically susceptible animals such as IL-$10^{-/-}$, IL-$2^{-/-}$, or TCR$\alpha\beta^{-/-}$ mice develop IBD-like colitis within weeks of birth but remain colitis-free when raised under germ-free conditions. When germ-free animals are reconstituted with normal microbiota or a few nonpathogenic species, rapid onset of disease occurs. Remarkably, mono-association of germ-free IL-$10^{-/-}$ mice with single microbial species resulted in different phenotypes depending on the type of bacterium. For example, *Enterococcus faecalis* caused a slow but severe distal colitis with additional upper GI manifestations, whereas *E. coli* caused more rapid but moderate cecal colitis with severe reactive atypia (Kim et al. 2005). Similarly, use of different antibiotics in these animal models led to different disease activities in various segments of the colon. This observation puts additional emphasis on the following question: which microbes are present in the diseased gut?

The immune system of IBD patients also shows evidence for a specific immune response targeted against the microbiota. Increased antibody titers are reported for anti-Saccharomyces cerevisiae mannan antibodies (ASCA) and certain flagellins in Crohn's disease patients and perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) in patients with ulcerative colitis. Interestingly, ASCAs were detected in the serum of Crohn's disease patients years before clinical diagnosis (30 % seropositivity vs. 0 % in the control group) (Israeli et al. 2005). Thus, even before obvious onset of disease or in healthy relatives of IBD patients, testing for these and other seromarkers may shorten time to diagnosis and enable more rapid and effective treatment. Duchmann et al. even proposed a loss of tolerance to autologous flora as a major mechanism of disease. They reported

proliferation of lamina propria mononuclear cells in response to autologous stool sonicates in IBD patients but not healthy controls (Duchmann et al. 1995). However, these findings could not be reproduced by other groups. Still, inappropriate immunoglobulins and T cell responses against microbial proteins and cell wall components have been found in feces, mucosa, and serum. As these responses seem to be specific to a small number of enteric antigens only, they suggest that some microbes may be more involved than others (Brandwein et al. 1997).

Microbes as Causative Agents in IBD

Several microbes have been accused of actually causing IBD as an infective agent. Most importantly is Mycobacterium avium paratuberculosis (MAP), which can invade epithelial cells and persist in macrophages. However, MAP has been isolated or detected in both healthy and diseased patients, and it could well be a regular part of our gut microbiota that is often missed due to limited sequencing techniques. Another more recently described bacterium is adherent-invasive *E. coli* (AIEC), which has been found in 25–35 % of patients with CD ileitis. AIEC adheres via CEACAM6, an enterocyte surface antigen that is increased in inflammation. It is unclear whether it actually promotes disease or simply adheres more easily under inflammatory conditions.

Changes in Microbial Composition in IBD

Advances in culture-independent sequencing techniques have given rise to theories focusing on the composition of the microbiome as a whole. A loss of microbial diversity has been reported in up to 50 % for Crohn's disease and 70 % for ulcerative colitis patients. This includes a decreased diversity of the core microbiome, which carries the functionally most important genes (Willing et al. 2010), and a decrease of the metagenome of 25 % (Qin et al. 2010). Plausibly, this would be accompanied by loss of function and adaptability in the human host. To a lesser extent these effects have also been reported for other inflammatory diseases (e.g., C. difficile colitis), so specificity has to be questioned. Typical for IBD, however, seems to be a loss of group similarity: patients from IBD cohorts show less overlap than healthy subjects (Scanlan et al. 2006). This supports a multifactorial pathogenesis of IBD where different genetic backgrounds may lead to different changes in the microbiome. Also, CD microbial communities have been reported to be less stable over time. This might reflect disease activity and treatment efficacy. As abnormalities are greater during active disease than in remission (Scanlan et al. 2006).

Upon closer examination the microbial depletion takes place mainly in the phyla of Firmicutes (50-fold) and Bacteroidetes (300-fold), whereas the usually scarce Proteobacteria show an increase (Frank et al. 2007). Principal component analysis, an analysis tool which graphically compares the most prevalent components of microbial communities, has in fact repeatedly been able to determine distinct clusters of microbial composition for healthy, Crohn's disease, and ulcerative colitis patients. Interestingly, it has also been able to identify subsets of patients with correlation to abscess formation, ileal disease, young age at surgery, early recurrence, and response to specific treatments (Frank et al. 2007; Qin et al. 2010). This has been shown for both mucosal and fecal samples. It could thus be a valuable clinical tool for diagnosis and therapeutic management of patients. A gene chip for detecting the relevant bacteria has facilitated the transition to more extensive patient cohorts, reliable clinical correlation, and predictive exploitation of results. However, due to high cost, clinical application is not realistic, yet.

Balance Between Detrimental and Protective Bacterial Species

A more recent theory is that of an (un)balanced microbiome. Whereas in healthy individuals, the microbial community is thought to be in a state of in a state of balance between detrimental and protective bacterial species, overgrowth of detrimental microbes or loss of protective ones – for whatever reason – could contribute to inflammation. While microbial communities as a whole are still too complex to study, first experiments with biofilm simulation are on their way.

Gut Microbiome, Overview, Table 2 Detrimental species in IBD with observed pathomechanisms

Microbe	Phylum	Disease	Observed mechanisms
Segmented filamentous bacteria (SFB)	Spore formation, close relative to Clostridia	CD	Firm epithelial attachment
		EAE	Secretion of serum amyloid A (which activates dendritic cells and T_{H17} cells)
Enterotoxigenic *Bacteroides fragilis* (ETBF)	Bacteroidetes	CD, diarrhea	Secretion of BFT toxin (which causes actin restructuring, cell swelling, and loss of microvilli)
			Epithelial barrier damage, epithelial leakage, and microbial translocation
Bacteroides thetaiotaomicron	Bacteroidetes	CD	Paneth cell stimulation via TLR activation induces release of antimicrobial peptides, which kill Gram-positive bacteria via cell wall damage
Bacteroides vulgatus	Bacteroidetes	CD, UC	Unknown
		CD	
Bifidobacterium animalis		Experimental colitis	Elevated IL-12/23 p40 levels
Enterococcus faecalis	Firmicute, Gram-positive	CD, UC, pouchitis psoriasis	Early loss of epithelial barrier function and resistance via TLR2-mediated NFkB activation
			Stimulates TNF and IL-12p70 secretion from peritoneal macrophages
			Oligoclonal T cell expansion
Fusobacterium varium	Fusobacterium, Gram-negative, anaerobic	UC	Induces apoptosis in colonocytes
			Formation of ulcers and crypt abscesses, probably via butyric acid

Detrimental Species

Several microbes have been shown to have a detrimental effect on the host (Table 2). Segmented filamentous bacteria (SFB), spore-forming bacteria closely related to Clostridia, show firm epithelial attachment especially in the terminal ileum. Interaction with the epithelium leads to a pro-inflammatory host gene program that stimulates lamina propria DCs to induce Th17 differentiation of naïve $CD4^+$ -T cells. This mechanism has been held responsible in the observation that SFB-mono-colonized mice were protected from Citrobacter rodentium infection (Ivanov et al. 2009). However, SFB was also linked to colitis in a SCID transfer model (Stepankova et al. 2007). Thus, it seems to play a dual role in gut immune homeostasis. The likely involvement of Th17 cells in Crohn's disease and the primary location of SFB in the terminal ileum make their involvement in Crohn's disease a likely possibility.

Enterotoxigenic *Bacteroides fragilis* (ETBF) causes loss of epithelial barrier function. It expresses the enterocyte-damaging toxin BFT that damages the tight junction protein E-cadherin, followed by epithelial leakage and translocation of other microbes. The epithelial cells undergo actin restructuring leading to cell swelling and unraveling of apical membrane microvilli. Despite this damaging character, ETBF prolongs the cell life span by inhibiting apoptosis and has also been associated with cancer development (Sears 2009).

Bacteroides thetaiotaomicron has been detected in increased numbers in the mucosa of Crohn's disease patients. It stimulates Paneth cells, which then upregulate RegIIIγ (in mice) or HIP/PAP (in humans) and release antimicrobial peptides damaging the cell wall of Gram-positive bacteria (Vaishnava et al. 2008). This could be one of many reasons for the reduction of Firmicutes in IBD.

Bacteroides vulgatus can either promote or protect from colitis depending upon the host's genetic background. In an early UC model using carrageenan-fed guinea pigs, isolates from UC patients caused robust disease (Onderdonk et al. 1984). IL-$10^{-/-}$ mice, however, did not develop

Gut Microbiome, Overview, Table 3 Currently used probiotics and assumed mechanisms

Microbe	Phylum	Disease	Observed mechanisms
Faecalibacterium prausnitzii	Firmicute (Clostridium leptum group)	CD	Anti-inflammatory cytokine profile (IL-12/IFN-g decrease, IL-10 increase)
			Correction of dysbiosis
Lactobacilli	Firmicute	IBD	Reduction of bacterial adherence/translocation
		EAE	Reduction of IL-12/IFN- g, TNF, and IgG2a
		Autoimmune arthritis	IL-10-induced generation of regulatory $CD4^+$ T cells
			Correction of dysbiosis
			Resistance to pathogens (Salmonella)
Bacteroides fragilis	Bacteroidetes	Colitis	Induction of IL-10-producing $CD4^+$ T cells, possibly via $CD103^+$ dendritic cells
		EAE	Suppression of local IL-17 levels
VSL#3		Type 1 diabetes	Restoration of epithelial barrier function, bacterial adherence/translocation, and mucosal histology
		UC	DC activation and IL-10 production in $CD4^+$ Tregs
Saccharomyces boulardii		CD	Unknown
Nonpathogenic *Escherichia coli*	Proteobacterium	UC	Unknown

colitis in the presence of *B. vulgatus* mono-association. Also in IL-$2^{-/-}$mice *B. vulgatus* attenuated *E. coli*-induced colitis (Sellon et al. 1998; Waidmann et al. 2003). *Bacteroides vulgatus* may in fact show a rather typical colitogenic profile with different immunologic effects depending on host background and presence of other microbes, thus, illustrating the complexity of gut homeostasis.

Enterococcus faecalis causes severe duodenitis and distal colitis in IL-$10^{-/-}$ mice (Kim et al. 2005). Observation of oligoclonal T cell expansion suggests several antigens are involved. It also caused loss of intestinal barrier function and transepithelial resistance via a TLR2-mediated NFkB activation pathway (Steck et al. 2011). However, how these epithelial cell and adaptive immune responses are connected is still unclear.

Fusobacterium varium has been isolated from inflamed UC mucosa. Over 60 % of UC patients are seropositive for *F. varium*, and the titer correlates with disease severity and extension of involved mucosa. *F. varium* has been shown to induce apoptosis in colonic epithelial cells, and its supernatants were reported to cause ulcers, crypt abscesses, and inflammatory infiltration in mice within 24 h. The exact mechanism of these events is unknown (Ohkusa et al. 2003).

This list is by no means complete. Advances in sequencing techniques and cohort size will hopefully shed more light onto their role in IBD and give us ideas how to regulate them.

Beneficial Species

The beneficial species are listed in Table 3 and will be discussed below under Probiotics.

Gut Microbiome and Other Auto-inflammatory Diseases

The intestinal microbiota has also been associated with a series of other diseases beyond the boundaries of the gut. Most obvious is its involvement in obesity and the metabolic syndrome. The microbiota of obese people and mice shows a reduction in Bacteroidetes and increase in Firmicutes that were reversible by low calorie diet. Germ-free mice, which are generally protected from obesity, show an increase in body fat of up to 60 % in 14 days without higher food intake upon colonization with regular microbiota. Furthermore, there is

a connection between the microbiota and innate immunity. TLR5-deficient mice develop a full metabolic syndrome, which does not develop under germ-free conditions. Interestingly, microbial transfer is sufficient to transfer the disease to wild-type germ-free mice stressing the importance of the microbiome in this disease (Vijay-Kumar et al. 2010).

Type 1 diabetes mellitus also has been linked to the gut microbiota and its effect on innate immunity. Nonobese diabetic (NOD) mice insufficient in the TLR signaling molecule MyD88 show a different penetrance of disease depending on microbial colonization. Raised germ-free, these mice develop full blown disease, while no diabetes occurs under specific pathogen-free (SPF) conditions (Wen et al. 2008). Also, young children with a genetic predisposition, who later developed type 1 diabetes, showed a gradual decrease in microbial diversity before onset of disease (Giongo et al. 2011).

Similar reports have emerged for other autoimmune diseases. Various models of experimental autoimmune encephalomyelitis (EAE), arthritis, and systemic lupus erythematosus show varying penetrance and disease severity depending on microbial colonization. Germ-free EAE model mice had attenuated disease with lower dendritic cell and $CD4^+$-T cell activity and lower cytokine responses. All of which could be restored by mono-association with segmented filamentous bacteria (SFB) (Berer et al. 2011). In a model of autoimmune arthritis, SFB also restored full phenotype as well as T cell numbers and function, which were repressed under germ-free conditions (Wu et al. 2010). Interestingly, microbial colonization is pro-inflammatory in these above-mentioned disease models but anti-inflammatory in the $MyD88^{-/-}$ NOD mice. This stresses the complex regulatory effects of microbial communities and their dependence on underlying host immune defects.

According to the hygiene hypothesis, early exposure to bacteria, viruses, or fungi sets the repertoire for the immune system. A clean environment and consecutive lack of microbial contact could therefore explain a myriad of autoimmune/auto-inflammatory diseases from a Th1-driven disease (e.g., diabetes) to allergic diseases (Th2). However intriguing, this theory has been replaced. The "microbial hypothesis" postulates that an imbalance in the human microbiota, which may arise from environmental changes, could be responsible for altered immune regulation. An altered gut microbiota has been reported in asthma, food allergies, atopic eczema, and dermatitis. An overall lack of Bifidobacteria and Lactobacilli has also been associated with the development of allergies. Underlying risk factors seem to be early antibiotic use and dietary changes.

Lastly, microbes have also been linked to the pathogenesis of cancer. Mouse models reported protection from carcinogen-induced colorectal carcinoma under germ-free conditions, as well as an involvement of TLR adaptor molecule MyD88 (Uronis et al. 2009). In humans the correlation cannot clearly be drawn. Recently two independent studies have shown increased Fusobacterium spp. in colorectal tumor tissue. Fusobacterium is an invasive bacterium formerly associated with appendicitis (Kostic et al. 2011). Likewise, two species, *Neisseria elongata* and *Streptococcus mitis*, were increased in the saliva of pancreatic cancer patients. Still, a better mechanistic understanding of the relationship between microbiota, immune system, and carcinogenesis is needed before new therapeutic approaches can be established.

Probiotics

Treating a microbe-driven disease by adding regulatory microbes seems intuitively obvious. However, probiotics have been around for centuries, without much measurable success. With the new appreciation of microbial composition and growing knowledge of immune and microbial interactions, recent results are more focused and somewhat promising.

Several bacterial families and species have been examined for anti-inflammatory or regulatory properties (Table 3). Lactobacilli reduced bacterial adherence and translocation and

ameliorated mucosal histology in an IL-$10^{-/-}$ colitis model. They also partially corrected microbial dysbiosis and conveyed resistance to salmonella infection. The exact mechanism is unknown but seems to involve reduction of IL-12, IFN-γ, TNF-α, and IgG2a (Madsen et al. 1999). Lactobacilli also had beneficial effects in experimental models of multiple sclerosis and arthritis.

Bacteroides fragilis was shown to protect from experimental colitis and encephalomyelitis (EAE) via secretion of polysaccharide A (PSA). Possible mechanisms are activation of $CD103^+$ dendritic cells, induction of IL-10 producing $CD4^+$-T cells, and local IL-17 suppression (Mazmanian et al. 2008; Ochoa-Reparaz et al. 2010).

Faecalibacterium prausnitzii and an unknown metabolite induced a typical anti-inflammatory cytokine profile in experimental colitis, with low IL-12/IFN-γ and high IL-10 levels in vitro and in vivo. It not only attenuated TNBS colitis significantly but also corrected the microbial dysbiosis, thus demonstrating relevance for microbe-microbe interaction. Interestingly, systemic administration of the metabolite was equally effective suggesting a possible beneficial effect in other systemic immune disorders (Sokol et al. 2008).

VSL#3, a standardized mixture of eight live probiotics from the lactic acid bacteria group, had a restorative effect on epithelial barrier function, bacterial adherence, and translocation and mucosal histology in an IL-$10^{-/-}$ colitis model (Madsen et al. 2001). It has also been shown to ameliorate TNBS colitis and decrease insulitis, beta cell destruction, and insulin resistance in NOD mice. The involved mechanisms include inhibition of dendritic cells and increased IL-10 production in $CD4^+$ Tregs (Hart et al. 2004).

However, in human disease the effects of probiotics are not as straightforward. Most studies have been carried out in a laboratory setting. Probiotic therapy works in theory. Clinical studies, on the contrary, show mixed to poor results. A positive impact has been shown for nonpathogenic *E. coli* strain Nissle 1917. It was equivalent to mesalazine in preventing relapse in quiescent ulcerative colitis when administered rectally (Kruis et al. 2004). However, other studies have shown no effect in Crohn's disease. *Saccharomyces boulardii* increased the time of remission when administered in addition to mesalamine in Crohn's disease, but administration of VSL#3 or nonpathogenic *E. coli* had only minimal effects (Guslandi et al. 2000).

If intestinal microbiota does in fact play a major role in the pathophysiology of immune diseases, there is hope for effective, possibly physiologic, therapies. Deeper insights into the mechanisms towards an immunoregulatory environment or correction of dysbiosis may, in fact, bear valid therapeutic approaches. However, various studies have shown that strain specificity and host genetic background are important. Thus, it would be desirable to find the right bug for each genetic defect, as to avoid unintended effects in the host. Metabolites of *Faecalibacterium prausnitzii* and *Bacteroides fragilis* have been shown to work on their own in the intestine or through systemic administration, so despite discouraging results from human studies, current work on probiotic therapies may soon be productive.

Conclusions and Future Directions

The gut microbiome markedly enriches our genome and influences our whole body throughout life. Technical advances over the last two decades have increased our understanding of the microbiota and stressed its importance in both health and disease. Several immune-related disorders such as inflammatory bowel disease, diabetes, atopy, and cancer show shifts in microbial composition (dysbiosis). Combined with refined analyzing techniques, these observations have a realistic potential to be applied in clinical prognosis and therapeutic management. So far efforts to manipulate the microbiota and correct dysbiosis are still rudimentary, and promising results have been limited to animal models. However complex, our understanding of the mechanisms of host-microbe and

microbe-microbe interactions is progressing. In order to further appreciate the role of the microbiota in health and disease, technical approaches have to become more cost-effective and more easily accessible. Additionally, simulation and study of biofilms in mammals will open up a new level of host microbial research. Given the recent acquisition of new information and advancing techniques, future revelations promise to be inspiring and productive.

Cross-References

- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Immune-Mediated Mechanisms of the Metabolic Syndrome
- ▶ NF-κB
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Tregs in the Liver

References

Berer K, Mues M, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature. 2011;479(7374):538–41.

Brandwein SL, McCabe RP, et al. Spontaneously colitic C3H/HeJBir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora. J Immunol. 1997;159(1):44–52.

Crohn BB, Ginzburg L, et al. Landmark article Oct 15, 1932. Regional ileitis. A pathological and clinical entity. By Burril B. Crohn, Leon Ginzburg, and Gordon D. Oppenheimer. JAMA. 1984;251(1):73–79.

Duchmann R, Kaiser I, et al. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). Clin Exp Immunol. 1995;102(3):448–455.

Eckburg PB, Bik EM, et al. Diversity of the human intestinal microbial flora. Science. 2005;308(5728):1635–1638.

Frank DN, St Amand AL, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A. 2007;104(34):13780–13785.

Giongo A, Gano KA, et al. Toward defining the autoimmune microbiome for type 1 diabetes. ISME J. 2011;5(1):82–91.

Guslandi M, Mezzi G, et al. *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. Dig Dis Sci. 2000;45(7):1462–1464.

Hart AL, Lammers K, et al. Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut. 2004;53(11):1602–1609.

Hill DA, Artis D. Intestinal bacteria and the regulation of immune cell homeostasis. Annu Rev Immunol. 2010;28:623–667.

Israeli E, Grotto I, et al. Anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. Gut. 2005;54(9):1232–1236.

Ivanov II, Atarashi K, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009;139(3):485–498.

Kim SC, Tonkonogy SL, et al. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. Gastroenterology. 2005;128(4):891–906.

Kostic AD, Gevers D, et al. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome Res. 2011.

Kruis W, Fric P, et al. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. Gut. 2004;53(11):1617–1623.

Ley RE, Peterson DA, et al. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006;124(4):837–848.

Madsen KL, Doyle JS, et al. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology. 1999;116(5):1107–1114.

Madsen K, Cornish A, et al. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology. 2001;121(3):580–591.

Mazmanian SK, Round JL, et al. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453(7195):620–625.

Ochoa-Reparaz J, Mielcarz DW, et al. A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease. Mucosal Immunol. 2010;3(5):487–495.

Ohkusa T, Okayasu I, et al. Induction of experimental ulcerative colitis by *Fusobacterium varium* isolated from colonic mucosa of patients with ulcerative colitis. Gut. 2003;52(1):79–83.

Onderdonk AB, Steeves RM, et al. Adoptive transfer of immune enhancement of experimental ulcerative colitis. Infect Immun. 1984;46(1):64–67.

Qin J, Li R, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59–65.

Scanlan PD, Shanahan F, et al. Culture-independent analyses of temporal variation of the dominant fecal microbiota and targeted bacterial subgroups in Crohn's disease. J Clin Microbiol. 2006;44(11):3980–3988.

Sears CL. Enterotoxigenic *Bacteroides fragilis*: a rogue among symbiotes. Clin Microbiol Rev. 2009;22(2):349–369. Table of Contents.

Sellon RK, Tonkonogy S, et al. Resident enteric bacteria are necessary for development of

spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun. 1998;66(11):5224–5231.

Sokol H, Pigneur B, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA. 2008;105(43):16731–16736.

Sonnenburg JL, Angenent LT, et al. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? Nat Immunol. 2004;5(6):569–573.

Steck N, Hoffmann M, et al. *Enterococcus faecalis* metalloprotease compromises epithelial barrier and contributes to intestinal inflammation. Gastroenterology. 2011;141(3):959–971.

Stepankova R, Powrie F, et al. Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RBhigh CD4+ T cells. Inflamm Bowel Dis. 2007;13(10):1202–1211.

Uronis JM, Muhlbauer M, et al. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. PLoS One. 2009;4(6):e6026.

Vaishnava S, Behrendt CL, et al. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. Proc Natl Acad Sci U S A. 2008;105(52):20858–20863.

Vijay-Kumar M, Aitken JD, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010;328(5975):228–231.

Waidmann M, Bechtold O, et al. *Bacteroides vulgatus* protects against *Escherichia coli*-induced colitis in gnotobiotic interleukin-2-deficient mice. Gastroenterology. 2003;125(1):162–177.

Weisburg WG, Barns SM, et al. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173(2):697–703.

Wen L, Ley RE, et al. Innate immunity and intestinal microbiota in the development of type 1 diabetes. Nature. 2008;455(7216):1109–1113.

Willing BP, Dicksved J, et al. A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. Gastroenterology. 2010;139(6):1844–1854. e1841.

Wu HJ, Ivanov II, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity. 2010;32(6):815–827.

H

Hair Loss in Lupus Erythematosus

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Synonyms

Alopecia in systemic disease; Discoid scarring alopecia; Hair loss in SLE; Lupus hair loss

Definition

The clinical presentation of lupus erythematosus (LE) may be confined to cutaneous disease or may involve various internal organs. Alopecia may occur with cutaneous and/or systemic lupus erythematosus. Hair loss over the scalp may range from localized scarring type of alopecia to non-scarring hair loss.

Discoid Lupus Erythematosus

Epidemiology

According to the Gilliam classification for cutaneous LE, DLE is considered an LE-specific skin lesion under the chronic cutaneous LE (CCLE) category. It is the most common clinical presentation of CCLE and commonly affects the scalp.

In patients with DLE, the scalp may be involved in 34–60 % of cases (Wilson et al. 1992; De Berker et al. 1992) and may be the only site affected in 10 % (Sontheimer and Mccauliffe 2002). Scalp DLE can be purely cutaneous or may be associated with SLE. DLE lesions may be the first clinical manifestation of SLE in 23–28 % of cases (Sontheimer and Mccauliffe 2002; Ross et al. 2005). The age range is from 20 to 40 years with a female predilection (Ross et al. 2005). It is not common in children.

While it is considered uncommon for patients with DLE to manifest systemic disease (Vera-Recabarren et al. 2010), approximately 5–10 % of patients with DLE will progress to SLE (Parodi and Rebora 1997; Wallace 1993). Among patients with SLE, about 5 % will have DLE lesions at some point (Sontheimer and Mccauliffe 2002).

DLE may be confined locally to the head and neck area or may be generalized (disseminated) affecting areas above and below the neck. The more widespread form has greater risk of developing systemic lupus disease and higher possibility of more severe SLE manifestations (Cardinalli et al. 2000; Callen 1982). Likewise, initial localized disease becoming generalized has higher association with developing internal organ involvement.

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Pathogenesis

The exact etiology of DLE remains unknown. The etiopathogenesis likely involves the interplay of multiple factors such as genetic, environmental, and immunologic regulatory mechanisms.

In DLE, scarring of the hair follicle is considered to be due to a primary process with resultant destruction of follicular units. The inflammatory infiltrates are mainly found around the bulge region, the site of the epithelial hair follicle stem cells (Harries et al. 2009; Cotsarelis 2006). Inflammation localized over the bulge area contributes greatly to the irreversible follicular damage seen in the primary scarring alopecias such as scalp DLE (Harries et al. 2009).

Lymphocytes comprise the main inflammatory cell infiltrate. The North American Hair Research Society (NAHRS) consensus scheme categorizes DLE as a primary lymphocytic cicatricial alopecia. DLE lesions have predominant T lymphocytes as mediators of immunity. There are elevated levels of CD4+ and CD8+ as well as increases in CD3+ T cells in the dermal infiltrates of DLE (Xie et al. 2011). Higher numbers of cytotoxic T lymphocytes and type 1 interferon (IFN) have been associated with scarring DLE (Wenzel et al. 2005a). In contrast, there was reduction in the regulatory cytokines, transforming growth factor (TGF) beta and interleukin (IL)-10 in patients with DLE. These immunosuppressive cytokines are involved in T cell regulation with lower levels seen in other autoimmune diseases (e.g., multiple sclerosis) (Antiga et al. 2011).

In patients with disseminated chronic DLE, there were findings of increased percentage of skin homing CD25+ CLA+ CD8+ T cells (Wenzel et al. 2005b). These cells are also seen in scarring DLE where they are found increased in the epidermis and outer root sheath of the hair follicle. Other proinflammatory cytokines upregulated in DLE include tumor necrosis factor (TNF) alpha, interleukin (IL)-2, and IFN gamma (Toro et al. 2000).

Effector mechanisms such as Fas/FasL interaction may also be involved in the pathogenesis of DLE. Macrophages with FasL expression were found around hair follicles with resultant apoptosis in follicles in DLE. Fas/FasL action could lead to destruction of hair follicles seen in scarring DLE (Nakjima et al. 1997).

There is convincing data for the role of ultraviolet (UV) light in the pathogenesis of lupus erythematosus particularly for acute and subacute cutaneous lupus erythematosus (Sontheimer and Mccauliffe 2002). However, the association of UV as a possible antigenic stimulus in the development of DLE over relatively sun-protected hair-bearing scalp is less evident.

Other environmental factors such as nonspecific cutaneous trauma (Koebner phenomenon) and smoking have been associated with the development and persistence of DLE lesions, respectively (Sticherling 2010).

Clinical Features

Scalp sites more commonly affected are the vertex followed by parietal and temporoparietal areas (Ross and Shapiro 2008).

Patients with scalp DLE may be relatively asymptomatic or may have accompanying symptoms of pruritus, pain, burning sensation, and tenderness over the affected areas (Vera-Recabarren et al. 2010).

DLE is a chronic, long-standing disease with lesions that typically start as erythematous to violaceous well-defined macules or papules with scaling. As the disease progresses, there is expansion of lesions to larger erythematous plaques with thick adherent scales extending into the ostia of the hair follicles. Removal of the adherent scale reveals an undersurface with keratotic spikes as is also seen in psoriasis, which correspond to hyperkeratosis of the follicular infundibulum (Carpet tack sign) (Ross and Shapiro 2008).

The inflammatory changes of erythema and scaling may be more prominent centrally in DLE compared to other primary cicatricial alopecias where inflammation commonly occurs over the outer margins (Mirmirani 2008).

With progressive disease, lesions become flatter with less erythema and develop depressed atrophic scarring, telangiectasia, and depigmentation. In some individuals especially those with

darker skin color, the lesions may exhibit central areas of hypopigmentation and hyperpigmentation over the periphery. Mottled dyschromia may also occur in some patients. With the development of progressive irreversible scarring alopecia, the lack of follicular ostia becomes more apparent. In some cases there may be increased curl of hair shafts (torsion due to fibrosis within hair follicle). During active disease, the pull test is positive for anagen hairs (Shapiro 2002).

Pathology

In DLE the main injury is found in the basal layer of the epidermis. DLE of the scalp shows vacuolar interface change of the follicular epithelium mainly at the infundibular level of the hair follicle with scattered epidermal dyskeratotic keratinocytes (Stefanato 2010) and superficial and deep lymphocytic inflammatory cell infiltrate around blood vessels and appendageal structures. Other features include prominent hyperkeratosis and follicular plugging. There is loss of sebaceous epithelium. The adjacent epidermis may be atrophic with pigment incontinence and the dermis has mucin deposition (Stefanato 2010; Whiting 2001).

In late disease there is epidermal and follicular BMZ thickening, perifollicular concentric lamellar fibrosis (Stefanato 2010), and interfollicular dermal fibroplasia. Diffuse scarring of the dermis with loss of elastic fibers can be apparent on Verhoeff-van Gieson stain.

Direct immunofluorescence of lesional discoid scalp skin shows IgG or IgM with C3 mainly over the epidermal and follicular basement membrane zone deposited in a thick continuous band or in a granular type of deposition (Stefanato 2010; Sperling 2003). The deposition localizes on the upper dermal collagen fibers and lamina densa of the epidermal basement membrane zone. Older lesions which are more than 3 months in duration compared to those less than a month old when examined showed higher immunoreactant positivity at the DE junction [3]. Scalp DLE with positive DIF results range from 63 % to 100 % (Sontheimer and Mccauliffe 2002; Ross and Shapiro 2008).

Differential Diagnosis

DLE of the scalp has to be differentiated from other scarring alopecias. One of the main differentials is lichen planopilaris, another primary scarring alopecia. This disease may present similarly as alopecic patches with scarring. Commonly there is perifollicular erythema and scaling in contrast to DLE where there is more diffuse erythema. Pigmentary changes are more common in DLE compared to lichen planopilaris. A biopsy may be needed to confirm the diagnosis. Other primary cicatricial alopecias such as folliculitis decalvans may present with scarring patches without papules and pustules in the chronic stage. Another differential is central centrifugal cicatricial alopecia (CCCA). This disease however occurs over the central aspect of the scalp in individuals predominantly of African descent. Typically features of erythema and dyspigmentation and follicular plugging are found in DLE but less likely in CCCA (Sontheimer and Mccauliffe 2002; Ross and Shapiro 2008).

Non-scarring Alopecia

Aside from the scarring alopecia seen in scalp DLE, there are other hair loss manifestations associated with lupus erythematosus. These hair loss features are considered nonspecific to the LE disease and are non-scarring in nature.

Patients with SLE may undergo telogen effluvium (TE) where there is excessive hair shedding diffusely affecting the entire scalp. Hair fall in TE exceeds physiologic levels, with approximately 150–300 or more hairs being shed per day. TE coincides with flares of systemic lupus whereby the disease process interrupts the hair growth cycle causing hairs to prematurely enter the telogen phase. When the acute flare of SLE subsides, hair shedding typically lessens. However, it takes a long time period (6 months to 1 year) for the hairs to grow back to previous hair density. This hair loss condition is nonspecific for LE and may occur due to other conditions (e.g., thyroid disorders, medications, postpartum, major stress, major surgery) (Trueb 2010).

Another type of hair loss which may occur with LE is termed lupus hair. Clinically, hair

strands appear dry and fragile. Affected hair shafts easily break off after exiting the surface of the scalp. Scalp sites which are commonly involved are the frontal hairline and the lateral hair margins in an ophiasis pattern. Lupus hair is a non-scarring type of alopecia associated with increased systemic activity. Hair regrowth occurs following disease control (Trueb 2010).

Patchy non-scarring alopecia may develop in severe established SLE disease. Clinically, multiple partial alopecic patches occur in various areas of the scalp. Some lesions may have mild erythema. The affected hairs are composed of telogen hairs or dystrophic anagen hairs. Dystrophic anagen effluvium results from severe illness causing transient hair matrix shutdown. In some cases there may be resultant constricted hair shafts (Pohl-Pinkus constriction). When the disease activity subsides, there is regrowth of areas with hair loss (Trueb 2010).

There have been some reports of the occurrence of alopecia areata in patients with SLE. While their association remains unclear and may be coincidental, a clinical pattern of alopecia areata may be seen. When biopsied and evaluated with H&E as well as direct immunofluorescence, typical immunologic features of lupus are found. The term "alopecia areata-like DLE" (Werth et al. 1992) has been utilized in this setting.

Course and Prognosis

Treatment of scalp DLE is usually initiated with intralesional corticosteroids (triamcinolone acetonide 10 mg/cc for a maximum volume of 2 cc every month depending on extent) and superpotent topical steroids, often in liquid or foam. In addition, systemic therapy with antimalarials is important to consider in those patients with multiple active sites, as well as with active serology. Short courses of systemic steroids may be added depending on severity of the condition in an attempt to prevent permanent scars.

Scalp DLE is considered a chronic, progressive disease. Early treatment of limited localized disease may halt the scarring process. In most cases though the disease results in progressive irreversible scarring of the scalp unless the patient is started on a remittive agent such as hydroxychloroquine. A serious sequela of chronic DLE is the risk of development of squamous cell carcinoma (scar carcinoma). Hair loss which accompanies SLE typically follows the activity of the disease with hair regrowth reversal possible upon disease control (Sontheimer and Mccauliffe 2002; Ross and Shapiro 2008).

Cross-References

- ▶ Alopecia Areata
- ▶ Discoid Lupus
- ▶ Discoid SLE
- ▶ Environment and Autoimmunity
- ▶ Immunology of Alopecia in Autoimmune Skin Disease
- ▶ Skin in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Antiga E, Del Bianco E, Difonzo E, et al. Serum levels of the regulatory cytokines transforming growth factors beta and interleukin 10 are reduced in patients with discoid lupus erythematosus. Lupus. 2011;20:556–60.

Callen JP. Chronic cutaneous lupus erythematosus: clinical, laboratory, therapeutic and prognostic examination of 62 patients. Arch Dermatol. 1982;118:412–6.

Cardinalli C, Caproni M, Bernacchi E, et al. The spectrum of cutaneous manifestations of lupus erythematosus. Lupus. 2000;9:417–23.

Cotsarelis G. Epithelial stem cells. A folliculocentric view. J Invest Dermatol. 2006;126:1459–68.

De Berker D, Dissayeneka M, Burge S. The sequelae of chronic cutaneous lupus erythematosus. Lupus. 1992;1:181–6.

Harries M, Meyer K, Paus R. Hair loss as a result of cutaneous autoimmunity: frontiers in the immunopathogenesis of primary cicatricial alopecia's. Autoimmun Rev. 2009;8:478–83.

Mirmirani P. Cicatricial alopecia. In: McMichael M, Hordinsky M, editors. Hair and scalp diseases: medical, surgical, and cosmetic treatments. New York: Informa; 2008.

Nakjima M, Nakajima A, Kayagaki N, et al. Expression of Fas ligand and its receptor in cutaneous lupus: implication in tissue injury. Clin Immunol Immunopathol. 1997;83:223–9.

Parodi A, Rebora A. ARA and EADV criteria for classification of systemic lupus erythematosus in patients with cutaneous lupus erythematosus. Dermatology. 1997;194:217–20.
Ross EK, Shapiro J. Primary cicatricial alopecia. In: Blume-Peytavi U, Tosti A, Whiting D, Trueb R, editors. Hair growth and disorders. Germany: Springer; 2008.
Ross EK, Tan E, Shapiro J. Update on primary cicatricial alopecias. J Am Acad Dermatol. 2005; 53(1):1–37.
Shapiro J. Cicatricial alopecia's. In: Shapiro J, editor. Hair loss: principles of diagnosis and management of alopecia. London: Martin Duniz; 2002.
Sontheimer R, Mccauliffe D. Cutaneous manifestations of lupus erythematosus. In: Wallace DJ, Hahn BH, editors. Dubois' Lupus erythematosus. Philadelphia: Lippincott Williams and Wilkins; 2002. p. 574–610.
Sperling LC. Chronic cutaneous lupus erythematosus (discoid lupus erythematosus). In: Sperling LC, editor. An atlas of hair pathology with clinical correlation. New York: Parthenon; 2003. p. 109–14.
Stefanato C. Histopathology of alopecia: clinicopathologic approach to diagnosis. Histopathology. 2010;56:24–38.
Sticherling M. Chronic cutaneous lupus erythematosus. In: Hertl M, editor. Autoimmune diseases of the skin: pathogenesis, diagnosis, management. New York: Springer; 2010.
Toro J, Finlay D, Dou X, et al. Detection of type 1 cytokines in discoid lupus erythematosus. Arch Dermatol. 2000;136:1497–501.
Trueb R. Involvement of the scalp and nails in lupus erythematosus. Lupus. 2010;19:1078–86.
Vera-Recabarren M, Garcia-Carrasco M, Ramos-Casals M, Herrero C. Comparative analysis of subacute cutaneous lupus erythematosus and chronic cutaneous lupus erythematosus. Br J Dermatol. 2010;162: 91–101.
Wallace DJ. The relationship between discoid and systemic lupus erythematosus. In: Wallace DJ, Hahn BJ, Quismorio Jr FP, editors. Dubois' Lupus erythematosus. Philadelphia/London: Lea and Febiger; 1993. p. 310–2.
Wenzel J, Uerlich M, Worrenkamper E, et al. Scarring skin lesions of discoid lupus erythematosus are characterized by high numbers of skin homing cytotoxic lymphocytes associated with strong expression of type 1 interferon induced protein MxA. Br J Dermatol. 2005a;153:1011–5.
Wenzel J, Henze S, Brahler S, et al. The expression of human leukocyte antigen DR and CD25 on circulating cells in cutaneous lupus and correlation with disease activity. Exp Dermatol. 2005b;14:454–9.
Werth VP, White WL, Sanchez MR, Franks AG. Incidence of alopecia areata in lupus erythematosus. Arch Dermatol. 1992;128:368–71.
Whiting DA. Cicatricial alopecia: clinicopathologic findings. Clin Dermatol. 2001;19:211–25.
Wilson C, Burge SM, Dean D, Dawber RP. Scarring alopecia in discoid lupus erythematosus. Br J Dermatol. 1992;126:307–14.
Xie Y, Jinnin M, Zhang X, et al. Immunohistochemical characterization of the cellular infiltrate in discoid lupus erythematosus. Biosci Trends. 2011;5(2):83–8.

Hepatic Lymphatic System

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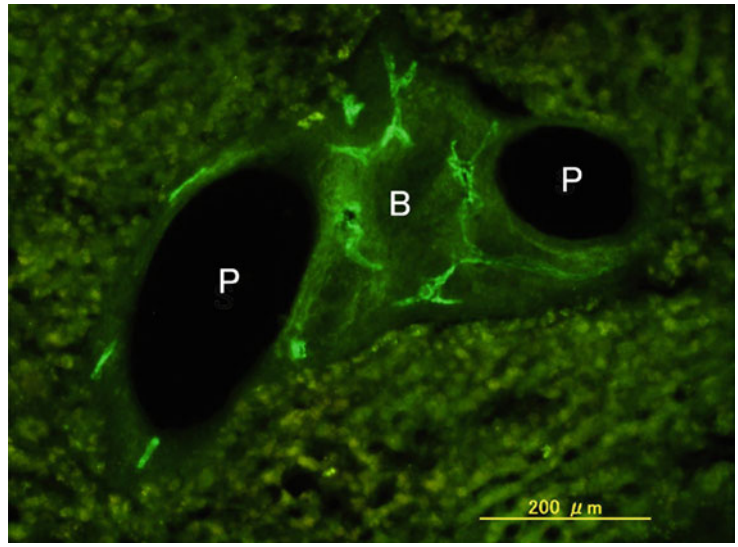
Synonyms

Lymph circulation of the liver

Definition (Overview)

As in other organs, the lymphatics in the liver function as a tissue drainage system and an immunological control system. The lymphatic vascular system consists of noncontractile initial lymphatics and contractile collecting lymphatics. Initial lymphatics are tubulosaccular and have many valves that allow unidirectional lymph flow. Basement membranes of the lymphatics are incomplete or absent. Reportedly, lymphatic basement membrane in human skin, digestive tract, and ovary contains laminin α4, β1, β2, γ1, type IV and XVIII collagens, and nidogen-1 (Vainionpää et al. 2007), but that in human liver remains to be studied. Lymphatic endothelial cells (LECs) show three types of intercellular contacts: end-to-end, overlapping, and interdigitated junctions. Open junctions are occasionally seen. Specialized junctional complexes, either fasciae occludentes or fasciae adherentes are seen in 65 % of the contacts. LECs are strongly attached at the anchoring filaments to the surrounding collagen and elastin fibers (Leak and Burke 1968). During expansion of the initial



Hepatic Lymphatic System,
Fig. 1 Fluorescent micrograph of the human liver showing lymphatics around the interlobular bile duct (*B*) and interlobular vein (or portal vein branch) (*P*). Immunohistochemistry to D2-40

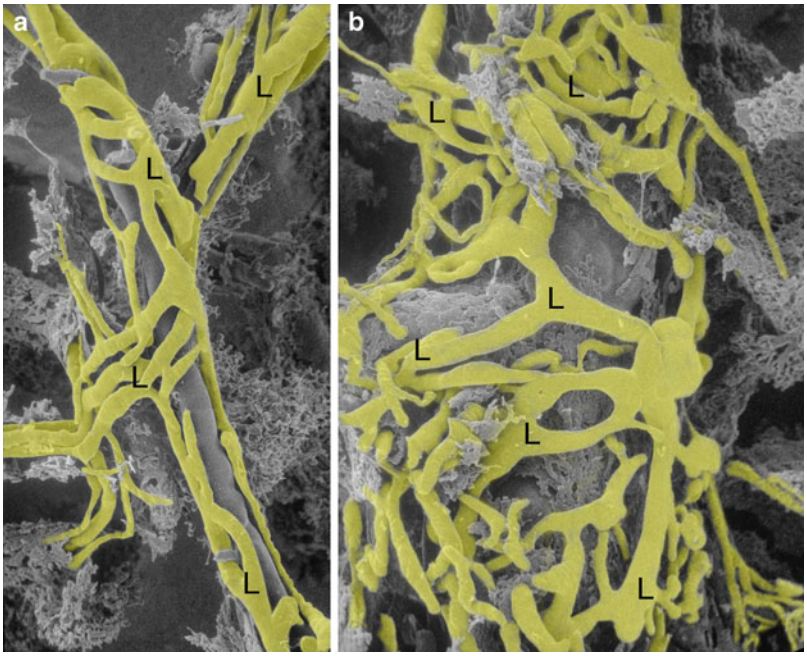
lymphatics, overlapping junctions can be opened, thus allowing fluid to flow from the interstitium into the lumen, while during compression, overlapping junctions can be closed, thereby retarding the backflow of lymph into the interstitium. Collecting lymphatics are, on the other hand, located downstream of the initial lymphatics and serve as a drainage system. Collecting lymphatics are endowed with smooth muscle cells and valves (Ohtani and Ohtani 2008b). Lymphatics in normal and cirrhotic human liver can be revealed using D2-40 (Fig. 1), a monoclonal antibody raised against a 40 kD membrane sialomucin (Breiteneder-Geleff et al. 1999). D2-40 specifically recognizes human podoplanin, which is homologous to human gp36 (Schacht et al. 2005). LYVE-1, a homolog of hyaluronan receptor CD44 (Jackson 2000), is expressed by hepatic sinusoids as well as by lymphatics (Carreira et al. 2001).

The liver produces a large volume of lymph, which is estimated to be 25–50 % of lymph flowing through the thoracic duct (Barrowman 1991). The hepatic lymph output is approximately half of the intestinal lymph output in sheep. The hepatic lymphatics fall into three categories depending on their locations: portal, sublobular, and superficial (or capsular) lymphatics (Lee 1923; Comparini 1969). It is suggested that 80 % or more of hepatic lymph drains into portal lymphatics, while the remainder drains through sublobular and capsular lymphatics (Popper and Schaffner 1957; Yoffey and Courtice 1970).

Because the liver is exposed to antigens from the gut or from the peripheral tissues, a considerable number of lymphocytes drain into the hepatic lymph nodes, which play a crucial role in immunosurveillance (Matsuno and Ezaki 2000; Xu et al. 2008). Lymphocytes and dendritic cells transmigrate from the blood into the portal tract (Matsuno and Ezaki 2000). These cells either remain in the portal tract or enter the draining lymphatics and migrate into the hepatic lymph node to contribute to the immune response. The minimal transit time of rat recirculating lymphocytes is 3–4 h in the liver and 5–8 h in the hepatic lymph nodes, in normal steady state (Xu et al. 2008).

Portal Lymphatics

Color gelatin injected into the portal vein first appears in the space of Disse; then reaches the perilobular space, i.e., the space of Mall; and finally enters portal lymphatics (Mall 1901). There are well-developed lymphatic networks around interlobular veins, arteries, and bile ducts, which extend distally as far as the terminal



Hepatic Lymphatic System, Fig. 2 Scanning electron micrographs of the corrosion cast of the rabbit liver showing lymphatic network (*L*, *yellow*) in the portal tract. The lymphatic network in the thin portal tract is composed by longitudinal vessels with transverse anastomoses (**a**). There is a dense lymphatic network in the thicker portal tract (**b**). The corrosion cast was made by retrograde injection of semipolymerized methacrylate into the bile duct

portal tract (Figs. 1 and 2). The lymphatics in the thin portal tract are composed of longitudinal vessels with transverse anastomoses (Fig. 2a). In the thicker portal tract, the lymphatics form dense networks (Fig. 2b).

The hepatic artery is responsible for 19 % of the hepatic blood flow in mice, 17 % in rats, 21 % in rabbits, 23 % in monkeys, 20 % in dogs, and 21 % in humans (Davies and Morris 1993) and primarily supplies the peribiliary plexus of the intrahepatic bile duct (Ohtani and Ohtani 2008a). Portal lymph comes from the plexus to some extent but Földi (1974) estimates that the plexus contributes less than 10 % to the total lymph output from the liver. However, 75 % of the blood in the liver comes from the portal vein, and almost all the blood of the liver flows through sinusoids. Furthermore, the protein concentration of hepatic lymph is about 80 % of the plasma protein concentrations (Yoffey and Courtice 1970). These suggest that lymph in the portal tract primarily derives from highly permeable sinusoids, i.e., hepatic sinusoids.

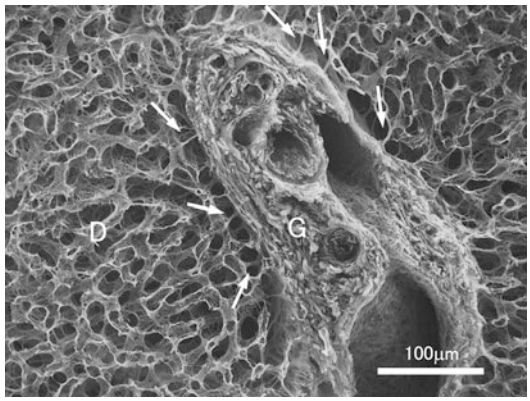
The space of Disse is continuous with the interstitial space of the portal tracts at the origin of the sinusoids (or inlet venule). There are also many channels with collagen fibers penetrating through the portal limiting plate to connect the space of Disse with the interstitial space of the portal tract (Ohtani et al. 2003). The density of the openings is approximately 1.3×10^3/mm (Ohtani et al. 2003).

The collagen fiber network in the liver has been demonstrated by NaOH maceration/scanning electron microscope technique (Ohtani 1988). There are condensations of collagen fibers in the Glisson's sheaths. Some collagen fibers in the portal tract extend along the inlet venules to continue with those in the space of Disse, while others travel independently of blood vessels through the channels with collagen fibers between the periportal limiting plate hepatocytes to connect with those in the space of Disse (Ohtani and Ohtani 2008a) (Fig. 3).

Horseradish peroxidase (HRP) injected into the blood vascular system of the rat appears in the hepatic sinusoids, in the space between limiting plate hepatocytes and in the space of Mall (Ohtani et al. 2003) (Fig. 4). This indicates that fluids in the space of Disse pass through channels between limiting plate hepatocytes to enter the space of Mall as well as through the space around the initial segment of the hepatic sinusoids (or inlet venules). Figure 5 shows pathways for

movement of fluid and cells from hepatic sinusoids to the portal lymphatics.

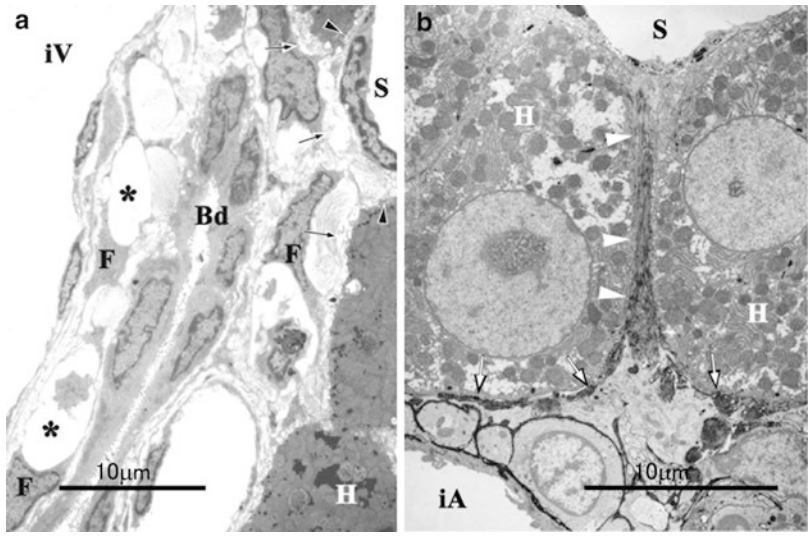
The interstitial space of the portal tract shows a porous structure partly lined with fibroblast-like cells (Fig. 4). The porous spaces frequently contain lymphocytes and/or dendritic cells. It is likely that the porous structures are prelymphatics (Ohtani et al. 2003) and serve as pathways for free cells such as lymphocytes and dendritic cells as well as fluid draining into the portal tract (Ohtani and Ohtani 2008a).



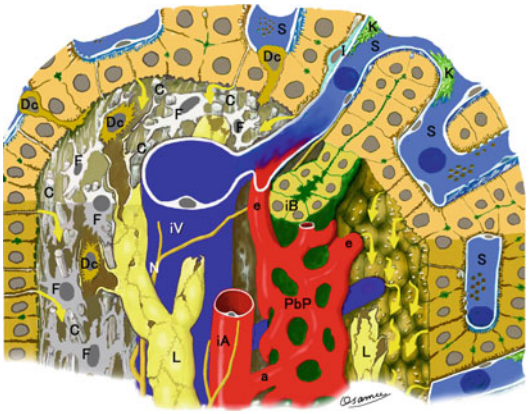
Hepatic Lymphatic System, Fig. 3 Scanning electron micrograph of collagen fiber network of the human liver. There is a condensation of collagen fibers in the Glisson's sheath (*G*). The collagen fibers in the space of Disse (*D*) form sheaths for housing the hepatic sinusoids. *Arrows* indicate collagen fibers passing through the layer of periportal limiting plate independently of blood vessels (From Ohtani 1988)

Sublobular Lymphatics

Sublobular lymphatics exist in many mammals, including rabbits, dogs, cats, and humans (Lee 1923; Yoffey and Courtice 1970), but not in smaller animals such as common tree shrews, rats, and mice. Sublobular lymphatics lead into lymphatics running along the inferior vena cava. Collagen fibers in the space of Disse are continuous with those around the central vein, which in turn increase in number toward the sublobular vein and the hepatic vein and finally continue into those around the vena cava (Ohtani and Ohtani 2008a). In addition, many collagen fibers traversing the hepatic limiting plate



Hepatic Lymphatic System, Fig. 4 Transmission electron micrographs of the intact (*A*) and HRP-injected rat liver. (**a**) *Arrowheads* indicate transition of the space of Disse at the initial segment of sinusoids (*S*) to the space of Mall (*arrows*). Processes of fibroblast-like cells (*F*) form spaces (*) that mimic lymphatic vessels, in which migrating cells (presumably dendritic cells and/or lymphocytes) can be seen. (**b**) HRP reaction products can be seen in the sinusoids (*S*), in the space of Disse, in the space (*arrowheads*) between limiting plate hepatocytes (*H*), and in the space of Mall (*arrows*). Also note collagen fibers with HRP in the space between limiting plate hepatocytes (From Ohtani et al. 2003)



Hepatic Lymphatic System, Fig. 5 A schematic representation of pathways of fluid and migrating cells such as dendritic cells (*Dc*) extending from the sinusoids (*S*) through the space of Disse, channels in the limiting plate, and interstitial space of the portal tract to portal lymphatic vessels (*L*). *Arrows* indicate the presumable flow direction. *C* collagen fibers, *iA* interlobular artery, *iV* interlobular vein, *iB* interlobular bile duct, *F* fibroblast, *I* Ito cell (or stellate cell), *K* Kupffer cell, *N* nerve, *PbP* peribiliary capillary plexus, *a* afferent vessel of PbP, *e* efferent vessel of PbP (From Ohtani et al. 2003)

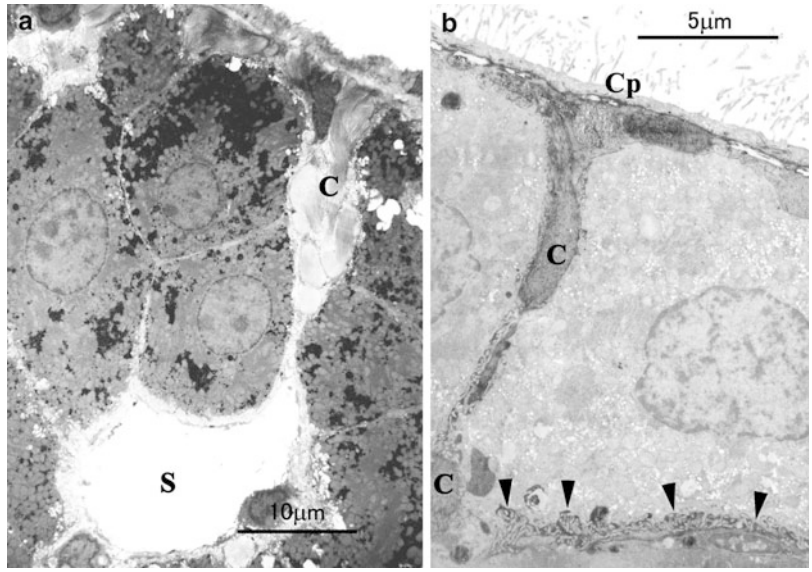
independently of blood vessels connect collagen fibers in the space of Disse with those around the sublobular veins. There are many channels with collagen fibers that pass through the limiting plate: the channels communicate the space of Disse to the sublobular interstitial space. HRP injected into the systemic vein flows along collagen fibers in the liver (Ohtani et al. 2003). In this context, it is likely that fluid in the space of Disse flows through the channels along collagen fibers traversing the hepatic limiting plate. Fluids in the space of Disse also flow through spaces along collagen fibers connecting those around sinusoids with central veins into the interstitial space of the sublobular and hepatic veins (Ohtani and Ohtani 2008a). Fluid in the interstitial space around the sublobular vein finally enters sublobular lymphatics.

Sublobular and portal lymphatics are sometimes enclosed in a common investment of connective tissue. The sharing of connective tissue of both kinds of lymphatics has been reported in rabbits, cats, dogs, and humans. In mice and rats, sublobular veins sometimes cross over interlobular veins. Liver lymph flow correlates directly with hepatic venous pressure in dogs and cats. Blood pressure of the portal vein is higher than that of the hepatic vein: normal blood pressure in the portal vein is 7 mmHg and that in the inferior vena cava is 2 mmHg. These facts suggest that some of the interstitial fluid in the portal tract may flow to the interstitial space around the sublobular veins to enter the sublobular lymphatics (Ohtani and Ohtani 2008a).

Superficial Lymphatics

According to Rusznyak and his colleagues (1967), superficial lymphatics in human liver form a very dense network, and their efferent vessels travel in several directions. Some of the lymphatics coming from the central area of the liver run in the falciform ligament toward the diaphragm; others pass downward into the lymph nodes of the porta hepatis. The lymphatics from the lateral area of the liver convexity advance in the triangular ligament toward the diaphragm and lead into the pancreaticolienal lymph nodes. The lymphatics in the coronary ligament drain into those along the inferior vena cava. The superficial lymphatics from the concave part of the liver curvature run in various directions toward their regional lymph nodes.

The space of Disse around the hepatic sinusoids close to the hepatic capsule communicates through the spaces containing collagen fibers between hepatocytes with the interstitial space of the hepatic capsule (Ohtani et al. 2003). HRP injected into the blood vascular system appears along collagen fibers running from the space of Disse through the space between hepatocytes to the interstitial space of the hepatic capsule (Fig. 6), suggesting that collagen fibers in the space of Disse are connected with those in the hepatic capsule (Ohtani et al. 2003). Thus, fluids in the hepatic sinusoids located close to the hepatic capsule can flow at least in part to the interstitial space of the hepatic capsule and finally enter the capsular lymphatics (Ohtani and Ohtani 2008a).



Hepatic Lymphatic System,
Fig. 6 Transmission electron micrographs of the intact (**a**) and HRP-injected (**b**) rat liver capsule. (**a**) Collagen fibers (*C*) running independently of blood vessels between hepatocytes connect those of the liver capsule (*Cp*) with those in the space of Disse (*arrowheads*). (**b**) HRP fills the space along the collagen fibers connecting those in the liver capsule with those in the space of Disse (From Ohtani et al. 2003)

Lymphatics in Pathological Conditions

Portal lymph flow increases in the liver with diffuse structural abnormalities such as fibrosis and cirrhosis. Lymph flows from the liver in cirrhotic rats are increased 30-fold, and hepatic lymph flows correlate well with portal venous pressure. As the hepatic sinusoids are capillarized in cirrhotic liver, the highly permeable blood-lymph barrier of the normal liver becomes markedly restrictive. The intravital fluorescent microscopy of CCl_4-induced liver fibrosis in the rat shows a strong negative correlation between the density of portal lymphatics and macromolecular trans-sinusoidal exchange. Lymphatics in the human liver also increase in size and number with the progression of chronic fibrosis and especially increase in end-stage cirrhosis necessitating transplantation.

The area of portal lymphatics increases in idiopathic portal hypertension (IPH), also known as Banti's syndrome, suggesting that the increased lymphatic area may be associated with a reduction in portal blood flow and increased lymph flow and that the latter may in turn reduce the high portal pressure in IPH.

Tumor metastasis to lymph nodes via lymphatics results in poor prognoses of patients with cancers. In human hepatocellular carcinoma (HCC) and some metastasized tumors, lymphatic vessel endothelial hyaluronan receptor (LYVE)-1- and Prox 1-positive lymphatics are abundant in the immediate vicinity of the tumors (Carreira et al. 2001). Poorly differentiated HCCs express vascular endothelial growth factor (VEGF)-C significantly stronger than well- or moderately differentiated HCCs, and the incidence of metastasis is higher in patients with VEGF-C-expressing HCC than those without. These seem to indicate that lymphangiogenesis is associated with enhanced metastasis as in other human cancer (Achen et al. 2005).

Conclusion

The hepatic lymphatic system falls into three categories depending on their location: portal, sublobular, and superficial lymphatics. The hepatic lymph primarily comes from the hepatic sinusoids, although the peribiliary capillary plexus contributes to the total hepatic lymph output to some extent (less than 10 %). Fluid filtered out of the sinusoids into the space of Disse flows through the channels traversing the limiting plate either independently of blood vessels or along blood vessels and enters the interstitial space of either the portal tract, sublobular veins, or the

hepatic capsule. Fluids and migrating cells in the interstitial space pass through prelymphatics to finally enter the lymphatics. The area of portal lymphatics increases in liver fibrosis and cirrhosis and in IPH. Lymphatics are abundant in the immediate vicinity of HCC and liver metastasis. HCC expressing VEGF-C is more liable to metastasize, indicating that lymphangiogenesis is associated with their enhanced metastasis. Little is known about the development of hepatic lymphatics in the fetal and early postnatal liver, which is a topic that warrants to be studied.

Cross-References

- ▶ Adaptive Immune Cells in the Liver
- ▶ Fibrosis
- ▶ Innate Immune Cells in the Liver
- ▶ Liver Vasculature and Microvasculature

References

Achen MG, McColl BK, Stacker SA. Focus on lymphangiogenesis in tumor metastasis. Cancer Cell. 2005;7:121–7.

Barrowman JA. Hepatic lymph and lymphatics. In: McIntyre N, Benhamou J-P, Bircher J, Rizzetto M, editors. Oxford textbook of clinical hepatology. New York: Oxford University Press; 1991. p. 37–40.

Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, Diem K, Weninger W, Tschachler E, Alitalo A, Kerjaschki D. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries. Podoplanin as a specific marker for lymphatic endothelium. Am J Pathol. 1999;154:385–94.

Carreira CM, Nasser SM, Tomaso ED, Padera TP, Boucher Y, Tomarev SI, Jain RK. LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis. Cancer Res. 2001;61:8079–84.

Comparini L. Lymph vessels in the liver in man. Angiologica. 1969;6:262–74.

Davies B, Morris T. Physiological parameters in laboratory animals and humans. Pharm Res. 1993;10:1093–5.

Földi M. Lymphgefäβsystem und Leber: funkutionelle und pathophysiologische Zusammenhänge. Leber Magen Darm. 1974;4:274–9.

Jackson DG. The lymphatics revisited: new perspective from the hyaluronan receptor LYVE-1. Trends Cardiovasc Med. 2000;13:1–7.

Leak LV, Burke JF. Ultrastructural studies on the lymphatic anchoring filaments. J Cell Biol. 1968;36:129–49.

Lee FC. On the lymph-vessels of the liver. Carnegie Inst Contrib Embryol. 1923;15:63–71.

Mall FP. On the origin of the lymphatics in the liver. Bull Johns Hopkins Hosp. 1901;12:146–8.

Matsuno K, Ezaki T. Dendritic cell dynamics in the liver and hepatic lymph. Int Rev Cytol. 2000;197:83–136.

Ohtani O. Three-dimensional organization of the collagen fibrillar network of the human and rat livers. Arch Histol Cytol. 1988;51:473–88.

Ohtani O, Ohtani Y. Lymph circulation in the liver. Anat Rec (Hoboken). 2008a;291:643–52.

Ohtani O, Ohtani Y. Organization and developmental aspects of lymphatic vessels. Arch Histol Cytol. 2008b;71:1–22.

Ohtani Y, Wang B-J, Poonkhum R, Ohtani O. Pathways for movement of fluid and cells from hepatic sinusoids to the portal lymphatic vessels and subcapsular region in rat livers. Arch Histol Cytol. 2003;66:239–52.

Popper H, Schaffner F. Liver structure and function. New York: McGraw-Hill; 1957.

Rusznyak I, Foldi M, Szabo G. Lymphatics and lymph circulation: physiology and pathology. 2nd ed. Oxford: Pergamon Press; 1967. p. 100–18.

Schacht V, Dadras SS, Johnson LA, Jackson DG, Hong Y-K, Detmar M. Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. Am J Pathol. 2005;166:913–21.

Vainionpää N, Bützow R, Hukkanen M, Jackson DG, Pihlajaniemi T, Sakai LY, Virtanen I. Basement membrane protein distribution in LYVE-1-immunoreactive lymphatic vessels of normal tissues and ovarian carcinomas. Cell Tissue Res. 2007;328:317–28.

Xu XD, Ueta H, Zhou S, Shi C, Koga D, Ushiki T, Matsuno K. Trafficking of recirculating lymphocytes in the rat liver: rapid transmigration into the portal area and then to the hepatic lymph. Liver Int. 2008;28:319–30.

Yoffey JM, Courtice FC. Lymphatics, lymph and lymphomyeloid complex. London: Academic; 1970.

IgA Nephropathy

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Definition

IgA nephropathy is a form of mesangioproliferative glomerulonephritis defined by the deposition of the immunoglobulin IgA within the mesangium of the glomerulus. This deposition is variably associated with mesangial hypercellularity and expansion of the mesangium.

Introduction and History

IgA nephropathy (IgAN) was first described in the 1960s by the French pathologist Jean Berger (1930–2011). He used the new technique of immunofluorescence to study renal biopsy specimens and noted that in a subset of patients with renal disease, deposits of the immunoglobulin IgA could be detected in the glomerulus. He published these findings in the seminal paper "Les dépôts intercapillaires d′IgA-IgG" in 1968 with Nicole Hinglais, an electron microscopist (Berger and Hinglais 1968). Since this time, IgAN, also known as Berger's disease, has become recognized as the commonest glomerulonephritis in countries where renal biopsy is routinely performed. Its defining feature is the finding of deposits of IgA in the glomerular mesangium. It is an important cause of chronic kidney disease with up to one-third of patients progressing to end-stage renal disease requiring dialysis or transplantation. Interestingly, there is a striking disparity between presentation, clinical course, and pathological findings.

Clinical Features

Presentation

The classic presentation of IgAN, recognized by Berger during his early studies, is of a young adult (often male) with visible hematuria coinciding with an upper respiratory tract infection (D'Amico 2004). Other mucosal infections, such as urinary or gastrointestinal infections, are also recognized triggers. Hematuria occurs within the first 48–72 h of the infection and clears within a few days. It may be associated with loin pain. Between episodes of visible hematuria, patients can have persistent non-visible hematuria. Thirty to forty percent of

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cases present in this way. A further 40 % of cases are identified incidentally by the presence of non-visible hematuria or proteinuria on routine urine dipstick testing. Five percent of cases present with the nephrotic syndrome (heavy proteinuria, hypoalbuminemia, edema). A smaller proportion present with acute kidney injury or hypertension.

Diagnosis and Investigations

The diagnosis of IgAN requires a renal biopsy (▶ Indications for Biopsy in Autoimmune GN). Supplementary tests should be carried out when there is a clinical suspicion of a secondary cause of IgAN (Pouria and Barratt 2008). Common secondary causes of IgAN include:

- Henoch-SchÖnlein purpura [▶ Vasculitis: Henoch-Schönlein Purpura]
- Liver cirrhosis, in particular alcoholic liver disease
- Celiac disease
- HIV infection

Further investigations, while not specific for IgAN, include routine monitoring of kidney function and urinary protein excretion and testing for generic complications of chronic kidney disease. Patients require long-term follow-up as up to one-third of patients will show progressive decline in kidney function requiring renal replacement therapy over 20–25 years. There are currently no tests at diagnosis which can predict accurately who will develop progressive disease.

Epidemiology

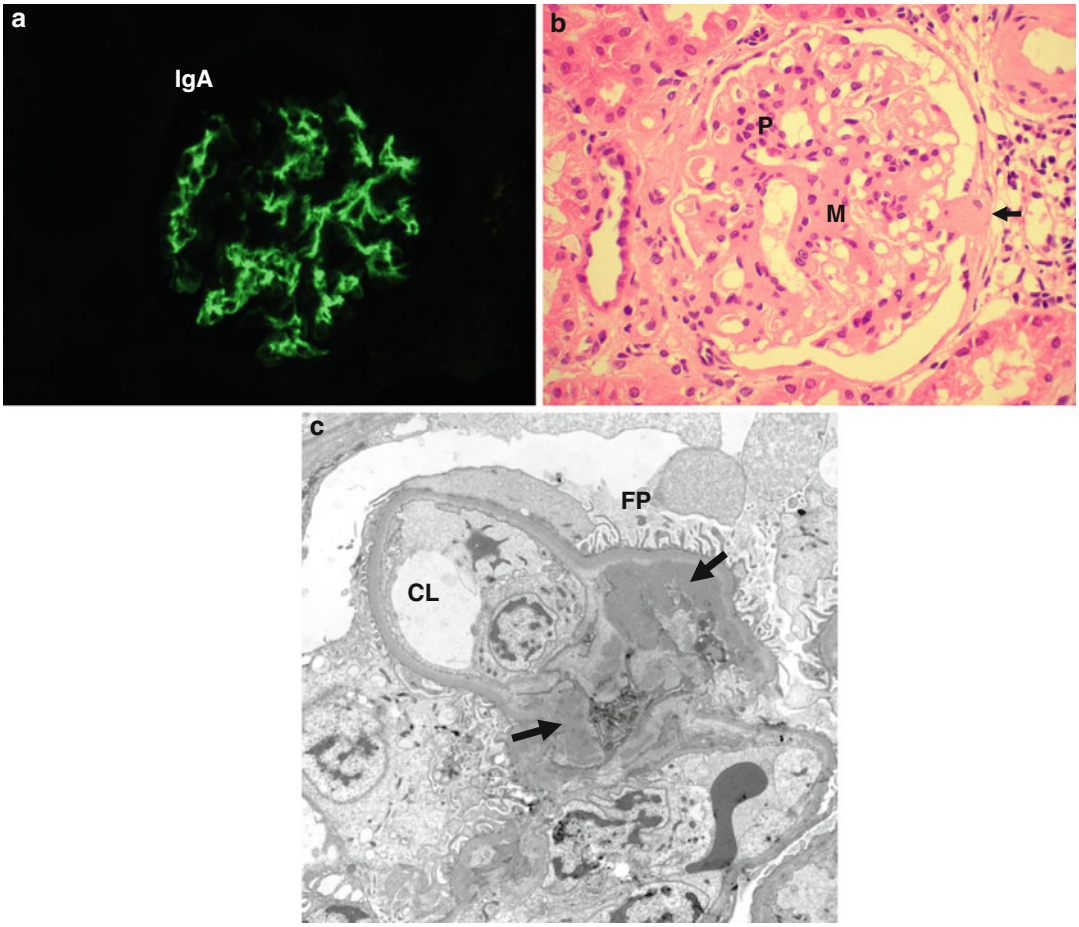
Peak incidence of IgAN is in the second and third decades, with a higher prevalence in men. The highest worldwide incidence is in Southeast Asia but this may reflect different approaches to investigation of renal disease and different thresholds for renal biopsy. IgAN is uncommon in African Americans. It is recognized that there may be many more undiagnosed asymptomatic cases, particularly in countries that do not screen for urine abnormalities and do not biopsy patients with minor urinary abnormalities.

Biopsy Findings and the Oxford Classification

The presence of dominant or codominant IgA deposits in the renal mesangium is the defining feature of IgAN. This is detected in renal biopsy specimens by immunofluorescence (Fig. 1a) or immunohistochemistry. In 15 % of cases, IgA is the only deposited immunoglobulin. Other immunoglobulins are also frequently detectable (IgG in 50–70 %, IgM in 31–66 %) but their presence does not appear to correlate with clinical outcome. The complement component C3 is also commonly present. IgA deposition is diffuse and global. Light microscopy appearances are, however, highly variable and do not correlate with degree of IgA deposition. The commonest appearance is of diffuse mesangial proliferation and extracellular matrix expansion (Fig. 1b) although focal changes may alternatively be seen. Progressive disease is associated with increasing accumulation of mesangial matrix. Crescents may be present. These occur most frequently in biopsy samples taken during episodes of macroscopic hematuria but can also occur in rare presentations with acute kidney injury.

Electron microscopy shows electron dense deposits in the glomerular mesangium (Fig. 1c). Deposits in glomerular capillary walls may also be seen and these can be subepithelial or, more commonly, subendothelial. Capillary loop deposits are associated with more severe disease. Glomerular basement membrane changes are seen in a minority but especially in those with heavy proteinuria or crescents.

The Oxford Classification of IgAN, published in 2009, is an international scoring system for evaluating pathological features on renal biopsy which can predict renal prognosis, independent of clinical and laboratory parameters (Cattran et al. 2009). Four variables are independently associated with risk of developing progressive disease: mesangial cell proliferation (M), endocapillary proliferation (E), segmental glomerulosclerosis (S), and extent of tubulointerstitial atrophy/interstitial fibrosis (T).



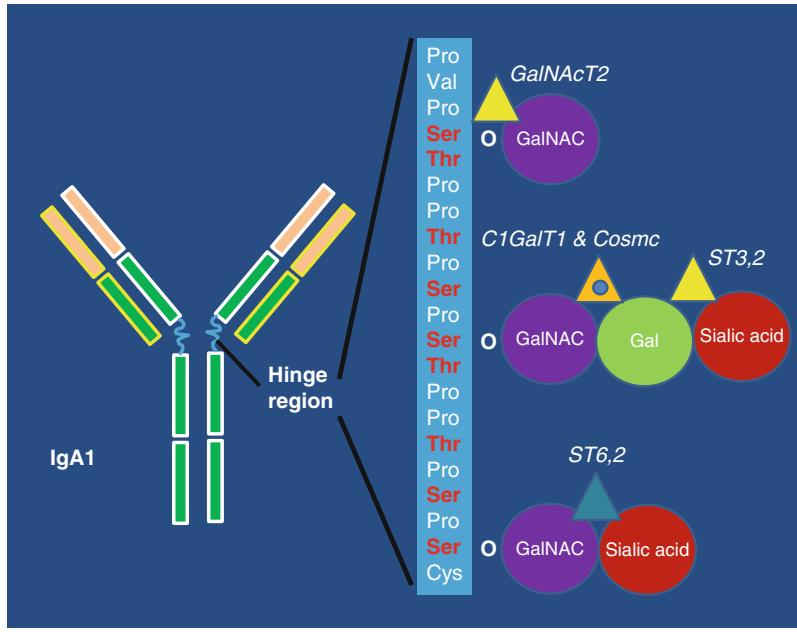
IgA Nephropathy, Fig. 1 (**a**) A renal biopsy showing immunofluorescent staining for IgA in a patient with IgA nephropathy (**b**) A renal biopsy showing mesangial proliferation (*P*) and expansion of the mesangial extracellular matrix (*M*) in a patient with IgA nephropathy (**c**) An electron micrograph of a portion of a glomerulus (*CL* capillary lumen, *FP* foot processes) showing electron dense immune complex deposits (*arrowed*) within the mesangium

Pathogenesis

Over the past 40 years, considerable progress has been made in characterizing a number of pathogenic pathways operating in IgAN, and this has been enhanced by an increased knowledge of the IgA immune system in health (Fig. 2). However, there is still a great deal we do not understand, and in particular it remains to be established whether IgAN is a single entity, or mesangial IgA deposition is simply the final common pathway for a number of distinct renal diseases (Barratt and Feehally 2011). Differences between the human IgA immune system and that of other species mean that, although animal models of IgAN exist, the extent to which they can be used to study mesangial IgA deposition is limited.

IgA in IgA Nephropathy

In humans IgA is the most abundant antibody and is predominantly present at mucosal surfaces and in secretions such as saliva and tears where it protects against mucosal pathogens (Brandtzaeg et al. 1999). IgA is also found in the systemic circulation, albeit at low levels. In humans,

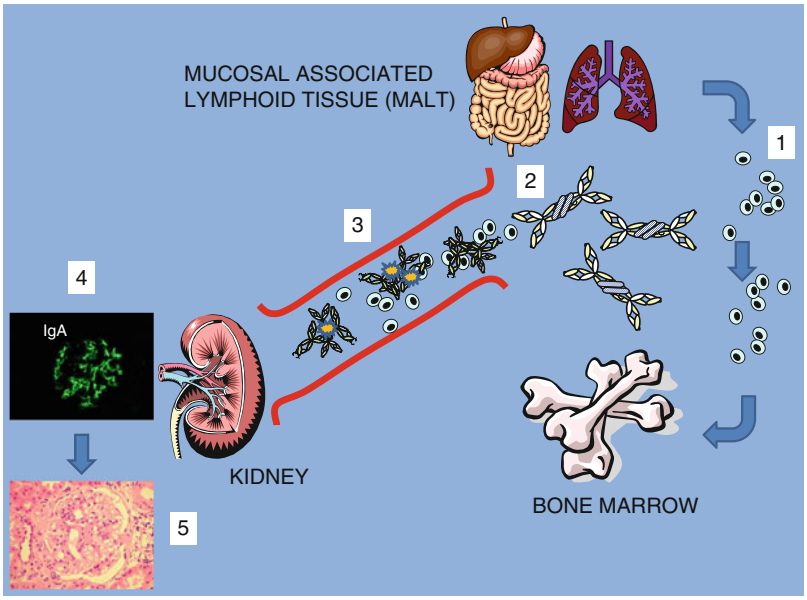


IgA Nephropathy, Fig. 2 *Proposed pathway for deposition of pathogenic IgA and renal injury*. There is a relocation of mucosa-derived polymeric IgA1 producing plasma cells from the mucosal associated lymphoid tissue (MALT) to the bone marrow (1). Mucosal-type IgA1 (polymeric, poorly galactosylated and low affinity) is secreted into the circulation (2). The stimulus for IgA secretion and the factors controlling its phenotype are unknown. Circulating IgA forms large immune complexes which include antibody-antibody complexes and complexes with the IgA Fc receptor CD89 (3). These immune complexes are prone to deposition in the renal mesangium (4). Mesangial deposition triggers the release of proinflammatory and profibrotic mediators from resident mesangial cells resulting in glomerular injury and scarring (5)

IgA exists in two isoforms, IgA1 and IgA2, which are secreted in varying ratios in a site-specific manner (Kerr 1990). IgA1 differs from IgA2 by the presence of a 17 amino acid hinge region. The hinge region is unusual in that it contains up to nine potential *O*-glycosylation sites, although current evidence would suggest that at most six of these sites can simultaneously carry *O*-glycans (Fig. 3). The IgA1 *O*-glycan chains are built-up in a step-wise fashion starting with *O*-linkage of N-acetylgalactosamine (GalNAc) to serine or threonine residues in the hinge. GalNAc may be further extended either by the addition of galactose (Gal) or sialic acid (N-acetylneuraminic acid, NeuNAc). Gal is β-1,3-linked to GalNAc by the enzyme core 1 β-1,3-galactosyltransferase (C1GalT1) and its molecular chaperone Cosmc which ensures its correct folding and stability. Alternatively, GalNAc may be sialylated in an α-2,6 linkage by the enzyme alpha-2,6-sialyltransferase. GalNAc-Gal may be further extended by sialylation of the Gal residue in an α-2,3 linkage by the enzyme alpha-2,3-sialyltransferase. Normal serum consists of a mixture of IgA1 *O*-glycoforms because of the heterogeneity afforded both by varied site occupancy and by occurrence of a mixture of GalNAc-Gal-NeuNAc structures at each of the *O*-glycosylation sites.

IgA is produced by IgA committed plasma cells. These cells reside principally in either the mucosa associated lymphoid tissue (MALT) or systemic sites such as the bone marrow. IgA has a characteristic phenotype dependent on its systemic or mucosal origin. In the systemic circulation, IgA is mostly IgA1, typically monomeric, of high affinity, and more heavily galactosylated IgA1 *O*-glycoforms predominate.

In mucosal secretions, IgA1 and IgA2 are present equally, IgA is predominantly polymeric,



IgA Nephropathy, Fig. 3 *O-glycosylation of IgA1 hinge region*. IgA1 contains a 17 amino acid hinge region which undergoes co/posttranslational modification by the addition of up to 6 *O*-glycan chains. These chains comprise *N*-acetylgalactosamine (GalNAc) in *O*-linkage with either serine or threonine residues. Galactose is β-1,3-linked to GalNAc by the enzyme Core 1 β-3-galactosyltransferase (C1GalT1) and its molecular chaperone Core 1 β-3-galactosyltransferase molecular chaperone (Cosmc) which ensures its correct folding and stability. Galactose may also be sialylated. In addition, sialic acid (*N*-acetylneuraminic acid, NeuNAc) may be attached directly to GalNAc by α-2,6 linkage which prevents further addition of galactose. *GalNAcT2* N-acetylgalactosaminyltransferase 2, *ST6,2* α-2,6-sialyltransferase II, *ST3,2* α-2,3-sialyltransferase 2

usually existing as J-chain-containing dimers, of lower affinity and poorly galactosylated IgA1 *O*-glycoforms are more common.

The IgA deposited in the kidneys in IgAN is of the IgA1 subclass and is predominantly polymeric. Another key feature of this deposited IgA is that it is enriched for poorly galactosylated IgA1 *O*-glycoforms (Allen et al. 2001). In parallel, most patients have elevated serum levels of poorly galactosylated IgA1 *O*-glycoforms, and this is thought to be the source of mesangial IgA (Moldoveanu et al. 2007). The reason for this change in the composition of circulating IgA1 *O*-glycoforms is not clear. Other serum *O*-glycosylated proteins display normal patterns of glycosylation in IgAN, suggesting any defect is specific to immunoglobulin *O*-glycosylation. Furthermore, IgD, the only other *O*-glycosylated immunoglobulin, also displays a normal pattern of *O*-glycosylation in IgAN. This suggests that changes in *O*-glycosyltransferase activity occurring after class switching from $sIgD^+$ naïve B cells are likely to be key to the changes in serum IgA1 *O*-glycoforms seen in IgAN. Current experimental work is therefore focussed on understanding the control of *O*-glycosylation during the class switch reaction and specifically in $sIgA^+$ antibody-secreting cells (ASC) and plasma cells. It is important, however, to appreciate that alteration in the composition of serum IgA1 *O*-glycoforms is neither an absolute requirement for the development of IgAN nor a ubiquitous finding in all patients. It is nevertheless the most consistent and reproducible finding in patient cohorts from North America, Europe, and Australasia.

Origins of Mesangial IgA

An increased understanding of the IgA immune system in health has shown that IgA production can be heavily influenced by the site of antigen encounter. At mucosal surfaces, antigen exposure

triggers an IgA immune response which is predominantly polymeric and low affinity. IgA responses in the systemic compartment result in monomeric high-affinity antibody production. Vaccination studies have also shown that IgA1 *O*-glycosylation varies depending on the site of antigen encounter. For instance, mucosal *Helicobacter pylori*-specific IgA1 antibodies are more likely to be poorly galactosylated compared to the IgA1 produced in response to systemic tetanus toxoid immunization. This pattern of differential *O*-glycosylation at mucosal versus systemic sites is also seen in patients with IgAN.

Many of the features of mesangial IgA are those typically seen in "mucosal-type" IgA. Overabundance of this "mucosal-type" IgA in the serum in IgAN might suggest that this IgA originates from mucosal sites. However, mucosal biopsies from patients with IgAN show significantly reduced numbers of polymeric IgA-secreting plasma cells compared with healthy subjects (Harper et al. 1994). This finding would suggest that mesangial IgA is not derived from the mucosal compartment. By comparison, increased numbers of polymeric IgA-secreting plasma cells are seen in bone marrow samples from patients with IgAN suggesting mesangial IgA is derived from systemically located $sIgA^+$ ASC and plasma cells (Harper et al. 1996). This has led to the hypothesis that in IgAN, mucosally primed $sIgA^+$ ASC relocate to systemic sites such as the bone marrow where they secrete their poorly galactosylated polymeric IgA1 directly into the circulation rather than into the submucosa for passage across the mucosal epithelium. In addition, the systemic microenvironment is likely to be very different from the MALT these plasma cells would normally inhabit, and it is possible that these plasma cells also receive cytokine signals promoting undergalactosylation of IgA1.

It is contended that one of the most likely mechanisms for this displacement is mishoming of mucosal $sIgA^+$ ASC to systemic sites. While there is emerging evidence of altered homing of B and T lymphocyte subsets in IgAN, much more work needs to be undertaken to fully evaluate this hypothesis.

Poorly Galactosylated IgA1 *O*-Glycoforms and IgA-Containing Immune Complex Formation

IgAN is being increasingly recognized as an immune complex deposition disease, although the trigger for immune complex formation is not known (Novak et al. 2008). Circulating immune complexes predominantly comprise polymeric IgA1 and immune complexes rich in poorly galactosylated polymeric IgA1 are believed to be both the source of mesangial IgA and the trigger for mesangial cell activation and induction of glomerular injury. IgA immune complexes containing poorly galactosylated IgA1 have been shown in vitro to bind to mesangial cells with high affinity and trigger mesangial cell proliferation and a proinflammatory and profibrotic phenotypic transformation similar to that seen in vivo. Understanding the reasons for IgA immune complex formation in IgAN is therefore of particular interest.

Poorly galactosylated IgA1 molecules have an increased tendency both to self-aggregate and form complexes with IgG antibodies. The absence of Gal appears to lead to exposure of hinge region epitopes that are normally sequestered, including terminal and sialylated GalNAc residues. There is now clear evidence that these epitopes, and particularly exposed GalNAc, are recognized by IgG and IgA1 antibodies with anti-glycan specificities (Suzuki et al. 2009). Furthermore, binding of glycan-specific IgG from patients with IgAN to poorly galactosylated IgA1 directly results in IgA-IC formation.

The IgA Fc receptor, CD89, has also been implicated in the development of circulating IgA immune complexes in IgAN (Boyd and Barratt 2010). CD89 is expressed by myeloid cells and two soluble receptor isoforms have been demonstrated to exist in vivo. The larger 50–70 kDa isoform has only been detected in patients with IgAN and has been demonstrated in IgA immune complexes in the circulation although not in the kidney. The smaller isoform is 30 kDa and, when complexed with IgA1, has been shown to protect against progressive renal disease, possibly through inhibition of immune complex formation. The role of CD89 in immune complex formation requires further investigation.

The Role of the Innate Immune System in IgA Nephropathy

There is accumulating evidence that in IgAN the innate immune system may play a significant role in driving pathogenic IgA production and immune complex formation. Toll-like receptors (TLR) play key roles in innate immunity to microbial pathogens via recognition of a diverse range of pathogen-associated molecular patterns (PAMPs). Of the 15 TLR thus far described, there is emerging evidence for contributions by TLR-4, TLR-9, and TLR-10 to the pathogenesis of IgAN.

Perhaps the two most intriguing observations to date are, firstly, that binding to B cell TLR leads to polyclonal activation of B cells, class switching, and immunoglobulin production. IgA secretion by mucosal lamina propria and tonsillar B cells is increased after TLR-9 stimulation implying that mucosal B cells can recognize PAMPs and secrete IgA in a T cell independent manner (Blaas et al. 2009). Secondly, ligation of B cell TLR-4 by bacterial LPS induces methylation of the Cosmc gene, leading to reduced activity of C1GalT1 and undergalactosylation of IgA1 (Qin et al. 2008). Whether B cell TLR-4 expression is increased in IgAN is not known; however, this in vitro data provides early evidence that mucosal pathogens might be able to influence the *O*-glycosylation of IgA antibodies.

Mechanisms of Glomerular Injury and Tubulointerstitial Scarring

What are the major pathogenic consequences of IgA deposition on mesangial cell, podocyte, and proximal tubular cell function in IgAN? The recently published Oxford Classification of IgA Nephropathy identified four key pathological consequences of IgA deposition that independently determine the risk of developing progressive renal disease: mesangial cell proliferation (M), endocapillary proliferation (E), segmental glomerulosclerosis (S), and tubulointerstitial scarring (T). There is increasing evidence, predominantly from in vitro models, that IgA immune complexes promote mesangial cell activation, resulting in proliferation (M) and release of proinflammatory and profibrotic mediators. These mediators, along with the direct effects of exposure to IgA immune complexes, cause podocyte injury, a process fundamental to segmental glomerular scarring (S) and proximal tubule cell activation, which drives tubulointerstitial scarring (T). Genetic factors are likely to play an important role in modulating all of these processes.

Genetics of IgAN

IgAN usually occurs sporadically; familial cases are rare. Multiple attempts have been made to establish a genetic basis for the disease both in familial cohorts and sporadic IgAN, although to date, little headway has been made in identifying the genes involved. There is, however, now evidence in several ethnic backgrounds to suggest that genetic factors heavily influence the composition of circulating IgA *O*-glycoforms in the serum (Gharavi et al. 2008). From these studies, it has also become apparent that first degree unaffected relatives of patients with IgAN often also have high levels of poorly galactosylated IgA1, supporting the hypothesis that changes in the composition of serum IgA1 *O*-glycoforms is only one part of a "multiple-hit" pathogenic process.

Several susceptibility loci have been identified from genome-wide association studies (GWAS) and one region that has gained particular interest is the major histocompatibility complex (MHC) (Feehally et al. 2010). Polymorphisms within the MHC have been associated with several autoimmune diseases, and an association in IgAN is consistent with an autoimmune component to IgAN, perhaps through production of autoantibodies with specificity for the poorly galactosylated IgA1 hinge region.

Treatment

No specific treatment for IgAN exists. An incomplete understanding of the pathological mechanisms causing pathogenic IgA production and mesangial deposition combined with the heterogeneity of observed pathological findings and clinical presentations has hindered attempts to find common targets for medical therapies.

Treatment strategies are therefore generic and aimed at ameliorating the downstream consequences of immune system activation and renal inflammation. In common with other causes of chronic kidney disease, management of patients with IgAN also involves treatment of secondary complications and in particular cardiovascular risk reduction (▶ Proteinuric Kidney Diseases: Importance of Blood Pressure Control).

Those with preserved renal function, non-visible hematuria, and <0.5 g/24 h proteinuria require no specific therapy. In patients with proteinuria >0.5 g/24 h, renin-angiotensin blockade has been shown to slow progression of IgAN. In those with ongoing proteinuria, despite maximal renin-angiotensin blockade, and continuing decline in renal function, there may be a role for immunosuppression. Published data in this patient group is, however, inconclusive and no clear guidance exists as to which agents should be used (Floege and Eitner 2010). Targeting immunosuppressive therapy to those patients exhibiting a pronounced inflammatory glomerular response may be more effective than treating patients whose renal biopsy shows predominantly fibrotic histological features. Such a strategy would optimize treatment benefits while limiting adverse effects of drugs which commonly have significant side effects. The recent publication of the Oxford Classification of IgAN which delineates specific pathological features associated with outcome may enable investigators to tailor treatment to patients based on degree of active glomerular injury.

Immunosuppression

The efficacy of corticosteroids in IgAN has been tested in several studies. Overall results have been equivocal and reports showing positive outcomes have been criticized for inadequate trial design and the presence of multiple confounding factors. The risks of high-dose corticosteroid use must also be considered. Similarly, studies examining the use of other immunosuppressive agents including azathioprine, cyclophosphamide, and mycophenolate mofetil have been inconclusive. There are large randomized controlled trials in progress in 2011 and hopefully the results when available will lead to a clearer understanding of the role of immunosuppression in the treatment of IgAN.

Fish Oil

The use of fish oil in IgAN has been advocated, although the evidence for benefit is again limited. Fish oil contains omega-3 fatty acids which have anti-inflammatory benefits, and while their use appears to be safe, it is limited by gastrointestinal side effects and fishy odor to breath and perspiration.

Tonsillectomy

The lymphoid tonsils contain an abundance of B cells and have been proposed to be a source of pathogenic IgA in IgAN. In patients where tonsillitis provokes visible hematuria, tonsillectomy has been shown to reduce episodes. There is conflicting evidence, however, as to whether tonsillectomy improves long-term renal prognosis, and given the significant morbidity associated with tonsillectomy, further clear evidence is needed before its use is embedded in standard clinical practice.

Rapidly Progressive Glomerulonephritis

In the small proportion of patients presenting with rapid loss of renal function and crescentic glomerulonephritis due to IgAN, treatment is in common with other forms of crescentic glomerulonephritis: high-dose corticosteroids, cyclophosphamide, and sometimes use of plasma exchange. However, the evidence base for this practice is limited and renal outcome is typically poor.

Renal Transplant

Patients with end-stage renal disease due to IgAN may be considered for transplantation. Recurrent IgA deposition is very common in transplanted kidneys; however, it rarely causes allograft failure (Floege 2004). At 5 years there is a 5 % risk of graft failure due to disease recurrence. In those who have lost a first graft due to recurrence, there is a 25 % risk of loss of subsequent grafts. No specific immunosuppression regimen has been shown to prevent IgA deposition and disease recurrence in IgAN.

Interestingly, when kidneys with subclinical IgA deposition have inadvertently been transplanted into patients without IgAN, deposited IgA immune complexes have cleared on rebiopsy. This supports the notion that mesangial IgA is derived from a circulating pool of pathogenic IgA and that the kidney has an ability to clear IgA deposits if given the opportunity.

Conclusion

IgAN is an important cause of renal disease which leads to end-stage renal failure requiring dialysis or kidney transplantation in a significant proportion of patients. Pathological features are disparate and the extent of IgA deposition does not correlate with degree of renal injury or clinical features. Additionally, despite extensive research into the disease, no unifying pathogenic mechanism can explain all the observed features. It is possible that several different pathways lead to the common finding of IgA in the renal mesangium, and specific combinations of risk factors influence disease progression and outcome.

Cross-References

- ▶ Indications for Biopsy in Autoimmune GN
- ▶ Proteinuric Kidney Diseases: Importance of Blood Pressure Control
- ▶ Vasculitis: Henoch-Schönlein Purpura

References

Allen AC, Bailey EM, Brenchley PE, Buck KS, Barratt J, Feehally J. Mesangial IgA1 in IgA nephropathy exhibits aberrant *O*-glycosylation: observations in three patients. Kidney Int. 2001;60(3):969–73.

Barratt J, Feehally J. Primary IgA nephropathy: new insights into pathogenesis. Semin Nephrol. 2011; 31(4):349–60.

Berger J, Hinglais N. Les depots intercapillaries d′IgA-IgG. J Urol Nephrol. 1968;74(9):694–5.

Blaas SH, Stieber-Gunckel M, Falk W, Obermeier F, Rogler G. CpG-oligodeoxynucleotides stimulate immunoglobulin A secretion in intestinal mucosal B cells. Clin Exp Immunol. 2009;155(3):534–40.

Boyd JK, Barratt J. Immune complex formation in IgA nephropathy: CD89 a ‘saint’ or a ‘sinner’? Kidney Int. 2010;78(12):1211–3.

Brandtzaeg P, Farstad IN, Johansen FE, Morton HC, Norderhaug IN, Yamanaka T. The B-cell system of human mucosae and exocrine glands. Immunol Rev. 1999;171:45–87.

Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, Troyanov S, et al. The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. Kidney Int. 2009;76(5): 534–45.

D’Amico G. Natural history of idiopathic IgA nephropathy and factors predictive of disease outcome. Semin Nephrol. 2004;24(3):179–96.

Feehally J, Farrall M, Boland A, Gale DP, Gut I, Heath S, et al. HLA has strongest association with IgA nephropathy in genome-wide analysis. J Am Soc Nephrol. 2010;21(10):1791–7.

Floege J, Eitner F. Combined immunosuppression in high-risk patients with IgA nephropathy? J Am Soc Nephrol. 2010;21(10):1604–6.

Floege J. Recurrent IgA nephropathy after renal transplantation. Semin Nephrol. 2004;24(3):287–91.

Gharavi AG, Moldoveanu Z, Wyatt RJ, Barker CV, Woodford SY, Lifton RP, et al. Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. J Am Soc Nephrol. 2008;19(5):1008–14.

Harper SJ, Pringle JH, Wicks AC, Hattersley J, Layward L, Allen A, et al. Expression of J chain mRNA in duodenal IgA plasma cells in IgA nephropathy. Kidney Int. 1994;45(3):836–44.

Harper SJ, Allen AC, Pringle JH, Feehally J. Increased dimeric IgA producing B cells in the bone marrow in IgA nephropathy determined by in situ hybridisation for J chain mRNA. J Clin Pathol. 1996;49(1):38–42.

Kerr MA. The structure and function of human IgA. Biochem J. 1990;271(2):285–96.

Moldoveanu Z, Wyatt RJ, Lee JY, Tomana M, Julian BA, Mestecky J, et al. Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. Kidney Int. 2007;71(11):1148–54.

Novak J, Julian BA, Tomana M, Mestecky J. IgA glycosylation and IgA immune complexes in the pathogenesis of IgA nephropathy. Semin Nephrol. 2008;28(1):78–87.

Pouria S, Barratt J. Secondary IgA nephropathy. Semin Nephrol. 2008;28(1):27–37.

Qin W, Zhong X, Fan JM, Zhang YJ, Liu XR, Ma XY. External suppression causes the low expression of the *Cosmc* gene in IgA nephropathy. Nephrol Dial Transplant. 2008;23(5):1608–14.

Suzuki H, Fan R, Zhang Z, Brown R, Hall S, Julian BA, et al. Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. J Clin Invest. 2009;119(6): 1668–77.

Immune Responses to the Hepatitis C Virus

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Synonyms

Non-A, non-B hepatitis (prior to 1989)

Definition

First identified in 1989, the hepatitis C virus (HCV) is the prototypical member of the Hepacivirus genus of the *Flaviviridae* family. HCV is a 9.6 kb positive-sense single-stranded RNA virus, which encodes a polyprotein cleaved to derive three structural and seven nonstructural proteins, as well as a frameshift protein. Despite its genetic and structural simplicity, the hepatitis C virus has nevertheless evolved remarkable capacity for persistent infection, with 70–80 % of affected individuals developing chronic infection. An estimated 170 million individuals are infected with HCV worldwide, with some 38,000 new infections occurring per year in the USA alone; there is no effective vaccine available against the virus. Cirrhosis occurs in approximately 20 % of persistently infected individuals, and up to 2.5 % develops hepatocellular carcinoma. The associated morbidity and mortality constitutes a major health burden globally; HCV-related liver disease is now the leading indication for liver transplantation in many countries (Bowen and Walker 2005a; Bowen et al. 2009) (▶ Liver Transplantation for Chronic Viral Hepatitis).

Introduction

Well-orchestrated responses mediated by innate, humoral, and cellular immune responses likely determine the outcome of HCV infection. A sizeable body of evidence indicates that both innate immunity and HCV-specific T cell immune responses are critical to control and clear HCV infection in the minority of individuals that resolve acute infection, while the role of HCV-specific antibodies remains unclear. Persisting HCV-specific T cell responses in the setting of chronic infection may, however, play a role in mediating ongoing hepatocellular injury associated with the eventual development of cirrhosis and its resultant complications.

Innate Immunity to HCV

Innate immunity is a critical component of the immune response to viral infections, both in terms of antiviral mechanisms within the infected cell and cellular mediators of the innate immune response such as NK cells and plasmacytoid dendritic cells (pDCs) (▶ Innate Immune cells in the Liver). Available evidence would suggest that HCV also elicits significant innate immune responses, which contribute to control of viral replication.

HCV is known to stimulate cellular pathogen-associated molecular pattern (PAMP) receptors (Gale and Foy 2005). Although HCV appears to have evolved mechanisms that subvert intracellular signaling via these molecules, as will be discussed below, interferon stimulated genes (ISGs) have nevertheless been shown to be upregulated in HCV-infected livers, both in acute and in chronic infection (Bowen et al. 2009). Both type I (IFN α and β) and type III (IFN-λ) interferons, produced as a component of innate immune responses, have been demonstrated to inhibit HCV replication in vitro (Chung et al. 2001; Marcello et al. 2006); consistent with this in vitro data, IFN-α has long been a central component of HCV antiviral therapy. Genetic evidence also points to an important role for innate immunity in HCV infection, with polymorphisms upstream of the IL28B (IFN-λ3) gene

correlating with both responses to IFN-α therapy, and with spontaneous clearance of viral infection (Shackel et al. 2010). Recent work in this field indicates that reduced likelihood of viral clearance in those individuals with certain variants in this genomic region may be associated with production of a newly described variant IFN-λ, IFNL4 (Prokunina-Olsson et al. 2013), potentially via reduction of response to type I and other type III interferons.

In addition to production of antiviral cytokines by infected cells, pDCs, the major source of type I IFNs, are able to produce these cytokines in response to HCV infection. This appears to be independent of their infection by HCV (Takahashi et al. 2010) and may be mediated via transfer of viral RNA from infected cells via endosomes, leading to triggering of toll-like receptor 7 (TLR7) (Dreux et al. 2012). pDCs have also been shown to produce type III IFN in response to HCV RNA in vitro (Stone et al. 2013). However, the role of pDCs in the control of HCV replication and resolution of acute infection remains to be determined.

NK cells both possess important direct antiviral functions via cytotoxicity and the production of antiviral cytokines, as well as by performing an important role in recruiting cells of the adaptive immune system to the infected liver (▶ Innate Immune cells in the Liver). Genetic evidence suggests a significant role for NK cells in resolution of HCV infection: expression of the inhibitory NK cell molecule killer immunoglobulin-like receptor (KIR) 2DL3 along with its ligand HLA-C group 1 is associated with increased likelihood of HCV clearance, potentially mediated via decreased NK cell inhibition mediated by this receptor combination (Bowen et al. 2009). A role for NK cells in HCV immunity is also suggested by data indicating that intravenous drug users repeatedly exposed to HCV who remain uninfected had a higher proportion of circulating mature NK cells than those exposed individuals who subsequently became infected (Golden-Mason et al. 2010). Similarly, a recently published report has described both NK and NKT cell activation in healthcare workers exposed to HCV who did not develop infection, indicating activation of the innate immune system by exposure to the virus, and a potential role for these cell populations in control of infection (Werner et al. 2013).

Humoral Immunity to HCV

Antibodies to HCV can develop as early as 6 weeks postinfection, although seroconversion more commonly occurs around 10–12 weeks postexposure. The antibody response to HCV is polyclonal and multi-specific. Both structural and nonstructural proteins are targeted (Bowen et al. 2009).

The role of the anti-HCV antibody response to HCV in controlling and resolving infection remains unclear. Early evidence indicated that changes in viral envelope sequences at the time of antibody seroconversion were associated with viral clearance, while stable viral quasispecies were associated with viral clearance (Farci et al. 2000). This data suggested that antibody responses play a role in HCV clearance and that escape from these responses is associated with viral persistence. The lack of in vitro models to study neutralizing antibody response to HCV hampered further studies until the development of recombinant HCV pseudoparticle systems. However, work using HCV pseudoparticles has led to variable conclusions. The majority of initial studies using HCV pseudoparticles found neutralizing antibodies to be rare in the setting of resolving acute infection, more often occurring in chronic infections, although this was not universally observed (Bowen et al. 2009). However, these studies involved the use of HCV pseudoparticles generated from prototypical viruses; in a study in which homologous viral pseudoparticles were utilized, clearance of infection was found to be associated with the development of neutralizing antibodies (Pestka et al. 2007).

Although the role of neutralizing antibodies in control of HCV infection remains unclear, it is apparent that at least in some circumstances, antibody responses are dispensable to viral clearance. Spontaneous clearance of HCV in the setting of hypogammaglobulinemia has been described

(Christie et al. 1997). HCV clearance has been demonstrated in chimpanzees in the absence of the development of a detectable antibody response, and in humans, viral clearance can occur in the absence of antibodies to structural proteins (Bowen and Walker 2005a). Furthermore, T cell responses have been described in likely exposed individuals who apparently cleared the virus without seroconverting or who did not mount antibody responses to structural proteins. In addition, preexisting antibodies do not provide protection from reinfection (Bowen et al. 2009).

Adaptive Cellular Immunity to HCV in Acute Infection

Effective HCV-specific cellular immune responses are critical for viral clearance. Failure to develop an HCV-specific CD4 T cell response is associated with viral persistence. In contrast, clearance of HCV during the initial acute infection is associated with a vigorous, broad CD4 T cell response (Bowen and Walker 2005a), with the initial presence and then loss of these responses also being associated with the development of chronic infection (Bowen and Walker 2005a; Schulze Zur Wiesch et al. 2012). The HCV-specific CD4 T cell response in individuals who successfully clear the virus is usually detectable within the first 3 months, peaking around 10 weeks; memory responses can remain detectable many years post-resolution (Bowen and Walker 2005a). Depletion of CD4 T cells in previously HCV-infected chimpanzees has been shown to lead to persistent infection upon repeat inoculation, confirming the critical role of CD4 T cells in controlling HCV infection (Grakoui et al. 2003).

In addition to broad, sustained CD4 T cell responses, CD8 T cell responses to HCV during the acute phase of infection are also required for viral clearance (Bowen and Walker 2005a; Rehermann 2009; Klenerman and Thimme 2012). However, although failure to develop strong acute CTL responses appears to be associated with progression to chronic infection, multi-specific CD8 T cell responses can also be detected in early infection in at least some individuals in whom viral persistence develops (Bowen and Walker 2005a; Kaplan et al. 2007). As observed with the CD4 T cell response, antiviral CD8 T cell responses remain detectable many years later in individuals who clear infection (Bowen and Walker 2005a). An unusual observation in HCV infection has been the delayed onset of interferon-γ (IFN-γ) production by virus-specific CD8 T cells detected early in infection; successful viral clearance can nevertheless ensure, and the significance of this observation thus remains uncertain (Bowen and Walker 2005a). The CD8 T cell response appears to peak later than the CD4 T cell response and continues to increase after falls in transaminases and decreases in viremia to undetectable levels, potentially indicating an ongoing suppressive response. Consistent with this, viremia does recur in some individuals following an apparent phase of viral control (Bowen and Walker 2005a). As observed with CD4 T cells, depletion of CD8 T cells in chimpanzees following an initial HCV infection was observed to be associated with persistence of viral replication until the recovery of detectable HCV-specific CD8 T cell responses (Shoukry et al. 2003).

The mechanisms of T cell control of HCV replication in vivo are incompletely understood. In a number of published studies, the onset of CD8 T cell responses correlated with serum transaminase increases and viral control (Bowen and Walker 2005a), suggesting that cytotoxic mechanisms may play a role in viral clearance. However, HCV infection has also been observed to resolve spontaneously without a significant transaminase increase and may correlate with increases in IFN-γ mRNA within the liver (Thimme et al. 2002), indicating that non-cytopathic control of viral replication may also lead to resolution of infection. Consistent with this hypothesis, in vitro evidence indicates that IFN-γ can inhibit HCV replication (Bowen and Walker 2005a; Jo et al. 2009). In vitro experiments comparing non-cytopathic to cytotoxic mechanism of HCV control have suggested that both pathways may be effective; however, non-cytopathic mechanisms were found to be more efficient, potentially indicating a predominant

role (Jo et al. 2009). However, it should be noted that as transaminase increases are a relatively crude tool in the assessment of hepatocellular injury, the alternative possibility exists that spontaneous resolution of acute hepatitis C without biochemical evidence of liver injury rather reflects cytolytic control of replication in a relatively small number of infected hepatocytes (Bowen and Walker 2005a); although the number of hepatocytes harboring HCV in the acutely infected liver is not known, and is likely highly variable, it may be as low as 5 % (Shoukry et al. 2004).

Adaptive Cellular Immunity to HCV in Chronic Infection

Studies of T cell responses in chronic infection have been complicated by the localization of HCV-specific T cells to the predominant site of infection, the liver. Thus, while a number of studies have detected low-frequency or narrowly focused HCV-specific CD8 T cell responses within the blood during chronic infection, others have isolated virus-specific CD8 T cells from the livers of chronically infected individuals, even after many years of infection (Bowen and Walker 2005a; Klenerman and Thimme 2012). In some individuals, such intrahepatic CD8 T cells can target multiple epitopes (Bowen and Walker 2005a; Klenerman and Thimme 2012). However, HCV-specific CD8 T cells from the livers of chronically infected individuals have also been described to express markers of apoptosis at high frequencies (Radziewicz et al. 2008) and, as will be discussed below, to possess phenotype associated with impaired function.

Studies in the chronic phase of HCV infection using functional methods of identification have demonstrated weak or absent CD4 T cell responses (Shoukry et al. 2004; Bowen and Walker 2005a). Several studies using non-functional methods of identification of virus-specific cells, such as CD25 or CD154 upregulation following stimulation, have suggested that virus-specific CD4 T cells are still present in chronic infection (Bowen and Walker 2005a; Mueller et al. 2010). In contrast, analyses using a limited range of MHC class II tetramers have also indicated that responses are poorly detectable in peripheral blood during chronic infection (Day et al. 2003; Ulsenheimer et al. 2006; Lucas et al. 2007). However, as with CD8 T cell responses, HCV-specific CD4 T cells have been identified in the livers of chronically HCV-infected individuals (Bowen and Walker 2005a), indicating that virus-specific CD4 T cells may also be sequestered to the liver in chronic infection. In some studies, virus-specific CD4 T cells persisting in chronic infection were found to elaborate altered cytokines patterns, including the anti-inflammatory cytokine IL-10 and impaired secretion of IL-2 (Bowen and Walker 2005a). Thus, although it would appear that HCV-specific CD4 T cell responses are relatively difficult to detect during chronic infection, the nature and function of these populations remains to be more fully delineated.

Mechanisms of Immune Evasion by HCV

How HCV establishes persistent infection remains poorly understood. However, as will be discussed below, the virus is postulated to subvert both innate and adaptive immune responses, facilitating its persistence in the majority of infected individuals.

Subversion of the Innate Immune Response by HCV: HCV has been demonstrated to interfere with IFN signaling, a critical component of the innate antiviral response. In in vitro experiments, HCV proteins have been shown to inhibit IFN signaling via the Jak-STAT pathway. The HCV protein NS5A has also been shown to induce IL-8, which inhibits IFN activity in vitro (Bowen et al. 2009). The HCV NS3/NS4A protease has been shown to inhibit production of type I IFN by infected cells, via cleavage of adaptor proteins in the signaling pathways of PAMP receptors. Replicating HCV RNA can be recognized by the intracellular PAMP receptor retinoic-acid-inducible gene I (RIG-I) and toll-like receptor-3 (TLR), a receptor for double-stranded RNA (Gale and Foy 2005). The HCV

protease NS3/NS4A inhibits the activation of interferon regulatory factor-3 (IRF-3), a transcription factor critical in type I IFN production, by cleavage of adaptor proteins in both of these pathways (Gale and Foy 2005; Bowen et al. 2009). However, other hepatotropic viruses that do not establish persistent infection have also been demonstrated to possess similar capacity (Chen et al. 2007; Yang et al. 2007); the extent to which this phenomenon plays a role in the establishment of persistent infection thus remains to be determined.

In addition to effects on intracellular signaling, HCV has also been demonstrated to potentially affect the function of DCs. Reduced numbers of pDCs have been reported in chronic HCV infection, although proportions may be greater than in acute infection. In vitro data suggests that HCV proteins can inhibit pDC function (Bowen et al. 2009; Rehermann 2009). It has been suggested that HCV can infect DCs, although this finding remains controversial (Bowen and Walker 2005a). HCV proteins have also been reported to affect DC function in vitro, as well as to influence antigen presentation by DCs in HCV-infected individuals (Bowen et al. 2009; Rehermann 2009). However, the results of studies of DC function in chronically HCV-infected subjects have been variable (Bowen and Walker 2005a), with no clear consensus as to whether function is impaired. Importantly, the immune defect in chronic HCV infection is virus specific in the absence of advanced liver disease. Therefore, although a role for inhibition of DC function in impaired initiation of adaptive immune responses remains a potential mechanism of HCV persistence, a long-lasting defect in DC function is not consistent with the clinical scenario observed in chronic infection.

HCV has also been shown to inhibit NK cell function via a range of mechanisms. In in vitro studies, the HCV E2 envelope protein was observed to downregulate NK cell function via binding to the tetraspanin CD81. In addition, HCV-derived peptide has been shown to bind to the NK cell inhibitory ligand HLA-E, resulting in impaired NK cell-mediated lysis (Bowen et al. 2009). NK cells isolated from HCV-infected individuals also exhibit reduced capacity to activate DCs (Bowen and Walker 2005a). Galectin-9, which is increased in chronic HCV infection (Mengshol et al. 2010), may also inhibit the function of NK cells (Golden-Mason et al. 2013). However, it remains unclear as to whether NK cells are dysfunctional in vivo during chronic HCV infection (Bowen et al. 2009).

Other Immunomodulatory Activities of HCV Proteins: HCV proteins may have direct effects on T cells. HCV core protein is amongst the best studied and is known to inhibit virus-specific T cell responses in vitro. This protein has been shown to bind to the Clq complement receptor, gC1qR, with subsequent inhibition of T cell proliferative responses, and may mediate immunomodulatory effects via this pathway (Bowen et al. 2009). In addition, the HCV core protein has been shown to impair function of the intrahepatic macrophage, the Kupffer cell (Tu et al. 2010). In vitro cross-linking of CD81 by the HCV E2 protein has been shown to lead to co-stimulation of T cells and may inhibit chemokine secretion and migration (Bowen et al. 2009). However, while such predominantly in vitro data indicates that HCV proteins may affect HCV-specific T cells, given the ubiquitous presence of these proteins, these interactions have the potential to influence all T cell responses. As noted previously, the lack of global immunosuppression in this infection is inconsistent with such a generalized mechanism (Bowen and Walker 2005a).

Impaired Function of HCV-Specific CD8 T Cells: CD8 T cell anergy, or functional silencing, has long been described in chronic viral infection in both animal models and in humans. Available data indicates that this situation can arise in the setting of a defective virus-specific CD4 T cell response (Bowen et al. 2009; Klenerman and Thimme 2012), not unlike that observed in HCV infection evolving toward chronic infection. Consistent with this, early studies of HCV-specific CD8 T cells in chronically infected individuals demonstrated phenotype characteristic of early stages of differentiation (Bowen and Walker 2005a).

Subsequent studies have revealed HCV-specific CD8 T cells to express low levels of CD127 and to upregulate expression of a variety of co-inhibitory markers, including programmed death-1 (PD-1), cytotoxic T lymphocyte antigen-4 (CTLA-4), 2B4, CD160, killer cell lectin-like receptor subfamily G member 1KLRG1, and T cell immunoglobulin domain and mucin domain 3 (Tim-3) (Klenerman and Thimme 2012). Expression of these co-inhibitory receptors is consistent with an exhausted phenotype, which is associated with chronic exposure to antigen. It thus remains unclear whether the observed characteristics of virus-specific CD8 T cells in chronic infection arise due to impaired initial priming, or as a consequence of ongoing exposure to cognate antigen. However, in vitro neutralization studies indicate that HCV-specific cellular immune function can be restored via inhibition of a number of these receptors, either alone or in combination (Klenerman and Thimme 2012). Despite this, a recent trial of monotherapy using a humanized monoclonal antibody against PD-1 in chronically HCV-infected individuals leads to transient reduction in only a minority of subjects (Gardiner et al. 2013), indicating that reversal of virus-specific cellular impairment by such inhibition is likely to require blockade of multiple inhibitory pathways.

Mutational Escape from Cytotoxic T Cell Responses: HCV is a highly mutable virus, as the NS5B RNA polymerase encoded by the HCV genome is error prone and lacks proof-reading capacity, leading to high base substitution rates. As such, it is susceptible to Darwinian selection pressures exerted by the adaptive immune response, which confers replicative advantages to viral subpopulations in which the genome encodes mutations that impair presentation or recognition of epitopes targeted by CD8 T cells. Such viral variants, termed "escape mutations," contribute to viral persistence as the affected CD8 T cell epitopes are no longer encoded by surviving viral populations. CD8 T cell escape mutations have been described as occurring early in HCV infectionm and correlate with the development of viral persistence in both chimpanzees and humans (Bowen and Walker 2005b; Bowen and Walker 2005c; Rehermann 2009; Klenerman and Thimme 2012). Epitope escape from CTL recognition may occur via a variety of mechanisms, including altered proteasomal processing leading to epitope destruction, abrogation of peptide/MHC binding, and altered recognition of variant peptide/MHC complexes by CD8 T cells (Bowen and Walker 2005b; Bowen and Walker 2005c; Rehermann 2009).

Not all epitopes targeted by CD8 T cells undergo mutational escape (Klenerman and Thimme 2012). Absence of escape mutation has been linked to the expression of an exhausted phenotype by CD8 T cells recognizing intact epitopes (Rutebemberwa et al. 2008), suggesting that these mechanisms may contribute to HCV persistence in concert. The parameters that govern the development of escape mutations are incompletely understood, but appear to comprise both viral and immunological factors. The development and persistence of CD8 T cell escape mutations are governed by the associated fitness cost to viral replication (Salloum et al. 2008; Uebelhoer et al. 2008; Dazert et al. 2009), which may impede their development in critical regions of the viral structure (Neumann-Haefelin et al. 2007), or necessitate the evolution of compensatory mutations within the viral genome that counteract effects on viral structure or function precipitated by escape mutations (Uebelhoer et al. 2008). The ability of HCV epitopes to escape CD8 T cell responses is also constrained by the characteristics of the T cell response. Escape mutations have been observed to occur in epitopes targeted by CD8 T cell responses comprised of relatively narrow T cell receptor (TCR) diversity (Meyer-Olson et al. 2004). In addition, the presence of "holes" in the clonal response to HCV-derived epitopes has also been described as favoring the emergence of escape mutations (Wolfl et al. 2008). Available data would also suggest that maintenance of effective CD4 T cell responses is important in protecting against the emergence of CD8 T cell escape mutations (Bowen and Walker 2005b, c).

Although a range of effects on HCV-specific CD4 T cell function have been described following in vitro stimulation with variant peptides

(Bowen and Walker 2005b, c), in contrast to CD8 T cell escape mutations, a similar phenomenon does not occur in epitopes targeted by CD4 T cell responses (Fleming et al. 2010; Fuller et al. 2010). It would thus appear that escape mutations do not play a significant role in the silencing of the virus-specific CD4 T cell response.

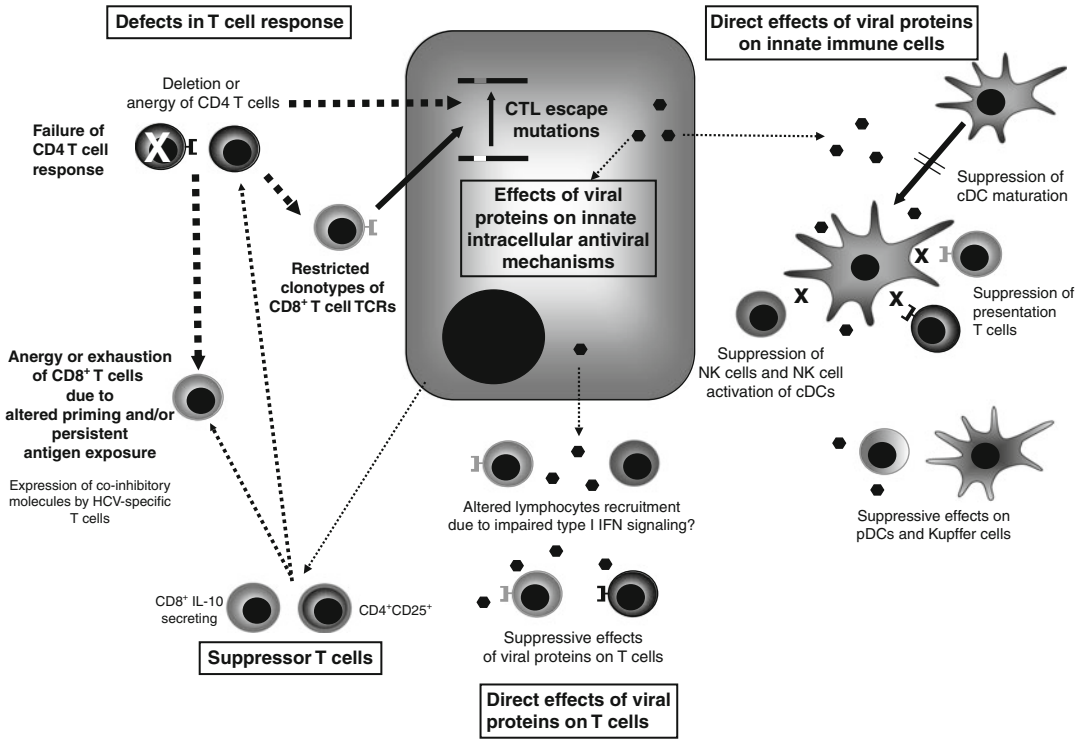
Regulatory T Cells: It has also been postulated that regulatory T cells (▶ Tregs in the Liver) modulate immune responses to HCV, thereby facilitating viral persistence. HCV-specific CD4 T cells secreting cytokines with regulatory effects, such as IL-10 and TGF-β, have been detected in chronically HCV-infected individuals (Bowen et al. 2009). CD4 Treg frequency in the peripheral blood during chronic HCV infection has been reported to be increased in some, but not all, studies (Bowen et al. 2009), while CD4 Tregs have been identified within portal infiltrates (Ward et al. 2007). It has been suggested that galectin-9, the expression of which is increased by Kupffer cells in HCV-infected liver, may mediate expansion of Tregs (Mengshol et al. 2010), although their function in the liver, and hence ability to inhibit the HCV-specific response, may be downregulated by PD-1-dependant mechanisms (Franceschini et al. 2009). In in vitro studies, CD4 Tregs from chronically HCV-infected individuals have been shown to suppress HCV-specific immune responses; however, responses to antigens derived from cytomegalovirus, Epstein-Barr virus, and influenza virus were also reduced (Bowen and Walker 2005a). In addition, similar findings were observed in chimpanzees that cleared HCV infection (Manigold et al. 2006). The role of Tregs in potentiating chronic HCV infection therefore remains unclear, although it may be that more selective effects on the HCV-specific immune response is conferred by increased frequencies of Treg populations within the liver during persistent HCV infection.

In addition to CD4 Tregs, CD8 T cells can also secrete regulatory cytokines with the ability to modulate the antiviral immune response. HCV-specific CD8 T cells that secrete IL-10, a cytokine with suppressive effects on the cell-mediated immune response, have been cloned from the livers of persistently HCV-infected subjects and directly identified within the liver parenchyma (Bowen et al. 2009; Rehermann 2009). Such IL-10 producing HCV-specific CD8 T cells have been demonstrated to suppress in vitro proliferative responses of liver-derived lymphocytes (Bowen et al. 2009). It has been suggested that this population of HCV-specific CD8 T cells may also contribute to viral persistence via impairing the antiviral functions of other virus-specific CD8 T cells.

A wide range of mechanisms have thus been postulated to facilitate evasion of the antiviral responses by HCV and facilitate chronic infection by this pathogen; these are summarized in Fig. 1. Although the level of evidence in support of these potential mechanisms is variable, it is apparent that the hepatitis C virus is able to persist despite the ongoing presence of detectable virus-specific CD8 T cells in many individuals with chronic HCV infection. It is probable that multiple pathways contribute to CD8 T cell failure, including viral escape from selection pressure and functional silencing of virus-specific CD8 T cell responses. Events during the acute phase of infection appear to play a significant role in determining the outcome of infection, pointing toward innate immunity and interactions of HCV with this arm of the immune response as a critical factor in the outcome of HCV infection. In addition, the indispensible role of virus-specific CD4 T cell responses in viral clearance may well be reflected in other phenomena associated with persistent infection, in particular the development of functionally impaired or exhausted CD8 T cell responses and the apparent emergence of CD8 T cell escape mutations in the absence of CD4 T cell help. Further investigations into innate immunity to HCV, the effects of HCV on T cell priming, and the evolution of the HCV-specific immune response will likely clarify the mechanisms underlying HCV persistence.

Pathogenesis of Liver Disease in Chronic Hepatitis C

The hepatitis C virus does not appear to be directly cytopathic. Transgenic expression of



Immune Responses to the Hepatitis C Virus, Fig. 1 Postulated mechanisms by which HCV evades the immune response and establishes persistent infection. Viral evasion of intracellular innate immune pathways may affect the ability of the infected cell to limit viral replication, initiate extracellular innate immune responses, and signal to HCV-specific immune cells. It is likely that impairment of both innate responses and virus-specific CD4 T cell responses is central to viral persistence, while ineffective virus-specific CD8 T cell responses may result from both the development of escape mutations and functional silencing. Modified from Bowen and Walker (Bowen and Walker 2005a)

the HCV polyprotein in mouse livers dose not lead to cytolysis, nor does the virus cause major cytopathic effects in cell culture (Bowen et al. 2009). Nevertheless, replication of HCV can cause endoplasmic reticular stress and oxidative stress (Choi and Ou 2006), which may contribute to hepatocellular injury and associated fibrosis (Tardif et al. 2005). In vitro evidence also suggests that proteins encoded by HCV may contribute to liver injury and fibrosis via a variety of pathways (Choi and Hwang 2006; Spengler and Nattermann 2007).

The contribution of the HCV-specific immune response to liver injury in chronic infection remains unclear. Although HCV-specific CD8 T cells are concentrated within the infected liver in persistent infection, it is thought that they comprise only a minority of the hepatic infiltrate in this condition (Abrignani 1997; Klenerman and Thimme 2012). In addition, studies correlating HCV-specific CD8 T cell responses with markers of hepatocellular injury have been inconsistent (Bowen and Walker 2005a). Nevertheless, this cellular population may play a role in the development of liver disease, mediating hepatocellular injury despite failure to effect viral clearance (Bertolino et al. 2002; Klenerman and Thimme 2012). Reactivation of HCV-related liver disease following withdrawal of immunosuppression and restoration of the host immune response is well documented and often associated with low or undetectable viremia, suggesting immune reconstitution as a cause (Bowen et al. 2009). CD8 T cells from HCV-infected individuals have been shown to mediate autologous hepatotoxicity and bystander killing

of non-antigen-expressing cells (Spengler and Nattermann 2007). Furthermore, Th1-type cytokine mRNA levels have been shown to correlate with the degree of liver fibrosis (Napoli et al. 1996), although the major cellular source of such cytokines has yet to be clarified. Production of the pro-fibrinogenic cytokine TGF-β by HCV-specific T cells has also been hypothesized to play a role in progressive liver injury, although recent in vitro studies have suggested a potential protective effect (Li et al. 2012). Other T cells contained within the immune infiltrate in persistently infected livers have also been suggested as mediators of ongoing injury, including CD161 expressing CD8 T cells via expression of IL-17 (Klenerman and Thimme 2012), and Tregs via production of IL-8 (Langhans et al. 2013). In addition, other cellular populations recruited to the liver during chronic inflammation, in particular macrophages, are also likely to play an important role in mediating hepatocellular injury and ensuing fibrosis (Bowen and Walker 2005a; Dolganiuc et al. 2007).

The pathogenesis of chronic hepatitis and associated fibrosis in chronic HCV infection are as yet not fully delineated. Although viral products may be directly involved in liver injury, persisting antiviral immune responses likely play a central role. However, the relative contributions of antigen-specific and antigen-independent mechanisms to hepatocellular injury in chronic HCV infection remain to be determined.

Conclusion

Despite its relative genetic and structural simplicity, the hepatitis C virus has evolved a remarkable propensity toward persistent infection. It is apparent that components of both the innate and adaptive immune responses are required to mediate viral clearance in the minority of infected individuals who clear HCV, with virus-specific cellular immune responses particularly implicated in resolution of infection. The means by which HCV escapes the immune response have not as yet been fully explained; however, a significant body of evidence now suggests that the virus is able to subvert both the innate and adaptive immune responses. It is hoped that ongoing research will further clarify both the mechanisms of viral clearance and interactions of the virus with the immune system that are central to its persistence, with the ultimate goal of developing a vaccine against this important human pathogen.

Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Adaptive Immune Cells in the Liver
- ▶ Innate Immune Cells in the Liver
- ▶ Liver Transplantation for Chronic Viral Hepatitis
- ▶ Tregs in the Liver

References

Abrignani S. Bystander activation by cytokines of intrahepatic T cells in chronic viral hepatitis. Semin Liver Dis. 1997;17:319–22.

Bertolino P, McCaughan GW, Bowen DG. Role of primary intrahepatic T-cell activation in the "liver tolerance effect". Immunol Cell Biol. 2002;80:84–92.

Bowen DG, Walker CM. Adaptive immune responses in acute and chronic hepatitis C virus infection. Nature. 2005a;436:946–52.

Bowen DG, Walker CM. Mutational escape from CD8+ T cell immunity: HCV evolution, from chimpanzees to man. J Exp Med. 2005b;201:1709–14.

Bowen DG, Walker CM. The origin of quasispecies: cause or consequence of chronic hepatitis C viral infection? J Hepatol. 2005c;42:408–17.

Bowen DG, Zekry A, Bertolino P, et al. Pathology and pathogenesis of hepatitis C. In: Dore G, Temple-Smith M, Lloyd A, editors. Hepatitis C: an expanding focus. Melbourne: IP Communications; 2009. p. 43–75.

Chen Z, Benureau Y, Rijnbrand R, et al. GB virus B disrupts RIG-I signaling by NS3/4A-mediated cleavage of the adaptor protein MAVS. J Virol. 2007;81:964–76.

Choi SH, Hwang SB. Modulation of the transforming growth factor-beta signal transduction pathway by hepatitis C virus nonstructural 5A protein. J Biol Chem. 2006;281:7468–78.

Choi J, Ou JH. Mechanisms of liver injury. III. Oxidative stress in the pathogenesis of hepatitis C virus. Am J Physiol Gastrointest Liver Physiol. 2006;290:G847–51.

Christie JM, Healey CJ, Watson J, et al. Clinical outcome of hypogammaglobulinaemic patients following

outbreak of acute hepatitis C: 2 year follow up. Clin Exp Immunol. 1997;110:4–8.
Chung RT, He W, Saquib A, et al. Hepatitis C virus replication is directly inhibited by IFN-alpha in a full-length binary expression system. Proc Natl Acad Sci USA. 2001;98:9847–52.
Day CL, Seth NP, Lucas M, et al. Ex vivo analysis of human memory CD4 T cells specific for hepatitis C virus using MHC class II tetramers. J Clin Invest. 2003;112:831–42.
Dazert E, Neumann-Haefelin C, Bressanelli S, et al. Loss of viral fitness and cross-recognition by CD8+ T cells limit HCV escape from a protective HLA-B27-restricted human immune response. J Clin Invest. 2009;119:376–86.
Dolganiuc A, Norkina O, Kodys K, et al. Viral and host factors induce macrophage activation and loss of toll-like receptor tolerance in chronic HCV infection. Gastroenterology. 2007;133:1627–36.
Dreux M, Garaigorta U, Boyd B, et al. Short-range exosomal transfer of viral RNA from infected cells to plasmacytoid dendritic cells triggers innate immunity. Cell Host Microbe. 2012;12:558–70.
Farci P, Shimoda A, Coiana A, et al. The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. Science. 2000;288:339–44.
Fleming VM, Harcourt G, Barnes E, et al. Virological footprint of CD4+ T-cell responses during chronic hepatitis C virus infection. J Gen Virol. 2010;91:1396–406.
Franceschini D, Paroli M, Francavilla V, et al. PD-L1 negatively regulates CD4+ CD25+ Foxp3+ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. J Clin Invest. 2009;119:551–64.
Fuller MJ, Shoukry NH, Gushima T, et al. Selection-driven immune escape is not a significant factor in the failure of CD4 T cell responses in persistent hepatitis C virus infection. Hepatology. 2010;51:378–87.
Gale Jr M, Foy EM. Evasion of intracellular host defence by hepatitis C virus. Nature. 2005;436:939–45.
Gardiner D, Lalezari J, Lawitz E, et al. A randomized, double-blind, placebo-controlled assessment of BMS-936558, a fully human monoclonal antibody to programmed death-1 (PD-1), in patients with chronic hepatitis C virus infection. PLoS One. 2013;8:e63818.
Golden-Mason L, Cox AL, Randall JA, et al. Increased natural killer cell cytotoxicity and NKp30 expression protects against hepatitis C virus infection in high-risk individuals and inhibits replication in vitro. Hepatology. 2010;52:1581–9.
Golden-Mason L, McMahan RH, Strong M, et al. Galectin-9 functionally impairs natural killer cells in humans and mice. J Virol. 2013;87:4835–45.
Grakoui A, Shoukry NH, Woollard DJ, et al. HCV persistence and immune evasion in the absence of memory T cell help. Science. 2003;302:659–62.
Jo J, Aichele U, Kersting N, et al. Analysis of CD8+ T-cell-mediated inhibition of hepatitis C virus replication using a novel immunological model. Gastroenterology. 2009;136:1391–401.
Kaplan DE, Sugimoto K, Newton K, et al. Discordant role of CD4 T-cell response relative to neutralizing antibody and CD8 T-cell responses in acute hepatitis C. Gastroenterology. 2007;132:654–66.
Klenerman P, Thimme R. T cell responses in hepatitis C: the good, the bad and the unconventional. Gut. 2012;61:1226–34.
Langhans B, Kramer B, Louis M, et al. Intrahepatic IL-8 producing Foxp3 CD4 regulatory T cells and fibrogenesis in chronic hepatitis C. J Hepatol. 2013;59:229–35.
Li S, Vriend LE, Nasser IA, et al. Hepatitis C virus-specific T-cell-derived transforming growth factor beta is associated with slow hepatic fibrogenesis. Hepatology. 2012;56:2094–105.
Lucas M, Ulsenheimer A, Pfafferot K, et al. Tracking virus-specific CD4+ T cells during and after acute hepatitis C virus infection. PLoS One. 2007;2:e649.
Manigold T, Shin EC, Mizukoshi E, et al. Foxp3+ CD4+ CD25+ T cells control virus-specific memory T cells in chimpanzees that recovered from hepatitis C. Blood. 2006;107:4424–32.
Marcello T, Grakoui A, Barba-Spaeth G, et al. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. Gastroenterology. 2006;131:1887–98.
Mengshol JA, Golden-Mason L, Arikawa T, et al. A crucial role for Kupffer cell-derived galectin-9 in regulation of T cell immunity in hepatitis C infection. PLoS One. 2010;5:e9504.
Meyer-Olson D, Shoukry NH, Brady KW, et al. Limited T cell receptor diversity of HCV-specific T cell responses is associated with CTL escape. J Exp Med. 2004;200:307–19.
Mueller M, Spangenberg HC, Kersting N, et al. Virus-specific CD4+ T cell responses in chronic HCV infection in blood and liver identified by antigen-specific upregulation of CD154. J Hepatol. 2010;52:800–11.
Napoli J, Bishop GA, McGuinness PH, et al. Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines. Hepatology. 1996;24:759–65.
Neumann-Haefelin C, Killinger T, Timm J, et al. Absence of viral escape within a frequently recognized HLA-A26-restricted CD8+ T-cell epitope targeting the functionally constrained hepatitis C virus NS5A/5B cleavage site. J Gen Virol. 2007;88:1986–91.
Pestka JM, Zeisel MB, Blaser E, et al. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. Proc Natl Acad Sci USA. 2007;104:6025–30.
Prokunina-Olsson L, Muchmore B, Tang W, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet. 2013;45:164–71.

Radziewicz H, Ibegbu CC, Hon H, et al. Impaired hepatitis C virus (HCV)-specific effector CD8+ T cells undergo massive apoptosis in the peripheral blood during acute HCV infection and in the liver during the chronic phase of infection. J Virol. 2008;82:9808–22.
Rehermann B. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. J Clin Invest. 2009;119:1745–54.
Rutebemberwa A, Ray SC, Astemborski J, et al. High-programmed death-1 levels on hepatitis C virus-specific T cells during acute infection are associated with viral persistence and require preservation of cognate antigen during chronic infection. J Immunol. 2008;181:8215–25.
Salloum S, Oniangue-Ndza C, Neumann-Haefelin C, et al. Escape from HLA-B*08-restricted CD8 T cells by hepatitis C virus is associated with fitness costs. J Virol. 2008;82:11803–12.
Schulze Zur Wiesch J, Ciuffreda D, Lewis-Ximenez L, et al. Broadly directed virus-specific CD4+ T cell responses are primed during acute hepatitis C infection, but rapidly disappear from human blood with viral persistence. J Exp Med. 2012;209:61–75.
Shackel NA, Bowen DG, McCaughan GW. Snipping away at hepatitis C. Hepatology. 2010;51:703–5.
Shoukry NH, Grakoui A, Houghton M, et al. Memory CD8+ T cells are required for protection from persistent hepatitis C virus infection. J Exp Med. 2003;197:1645–55.
Shoukry NH, Cawthon AG, Walker CM. Cell-mediated immunity and the outcome of hepatitis C virus infection. Annu Rev Microbiol. 2004;58:391–424.
Spengler U, Nattermann J. Immunopathogenesis in hepatitis C virus cirrhosis. Clin Sci (Lond). 2007;112:141–55.
Stone AE, Giugliano S, Schnell G, et al. Hepatitis C virus pathogen associated molecular pattern (PAMP) triggers production of lambda-interferons by human plasmacytoid dendritic cells. PLoS Pathog. 2013;9:e1003316.
Takahashi K, Asabe S, Wieland S, et al. Plasmacytoid dendritic cells sense hepatitis C virus-infected cells, produce interferon, and inhibit infection. Proc Natl Acad Sci USA. 2010;107:7431–6.
Tardif KD, Waris G, Siddiqui A. Hepatitis C virus, ER stress, and oxidative stress. Trends Microbiol. 2005;13:159–63.
Thimme R, Bukh J, Spangenberg HC, et al. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. Proc Natl Acad Sci USA. 2002;99:15661–8.
Tu Z, Pierce RH, Kurtis J, et al. Hepatitis C virus core protein subverts the antiviral activities of human Kupffer cells. Gastroenterology. 2010;138:305–14.
Uebelhoer L, Han JH, Callendret B, et al. Stable cytotoxic T cell escape mutation in hepatitis C virus is linked to maintenance of viral fitness. PLoS Pathog. 2008;4:e1000143.
Ulsenheimer A, Lucas M, Seth NP, et al. Transient immunological control during acute hepatitis C virus infection: ex vivo analysis of helper T-cell responses. J Viral Hepat. 2006;13:708–14.
Ward SM, Fox BC, Brown PJ, et al. Quantification and localisation of FOXP3+ T lymphocytes and relation to hepatic inflammation during chronic HCV infection. J Hepatol. 2007;47:316–24.
Werner JM, Heller T, Gordon AM, et al. Innate immune responses in hepatitis C virus exposed healthcare workers who do not develop acute infection. Hepatology. 2013 doi:10.1002/hep.26353 [Epub ahead of print].
Wolfl M, Rutebemberwa A, Mosbruger T, et al. Hepatitis C virus immune escape via exploitation of a hole in the T cell repertoire. J Immunol. 2008;181:6435–46.
Yang Y, Liang Y, Qu L, et al. Disruption of innate immunity due to mitochondrial targeting of a picornaviral protease precursor. Proc Natl Acad Sci USA. 2007;104:7253–8.

Immune System and Kidney

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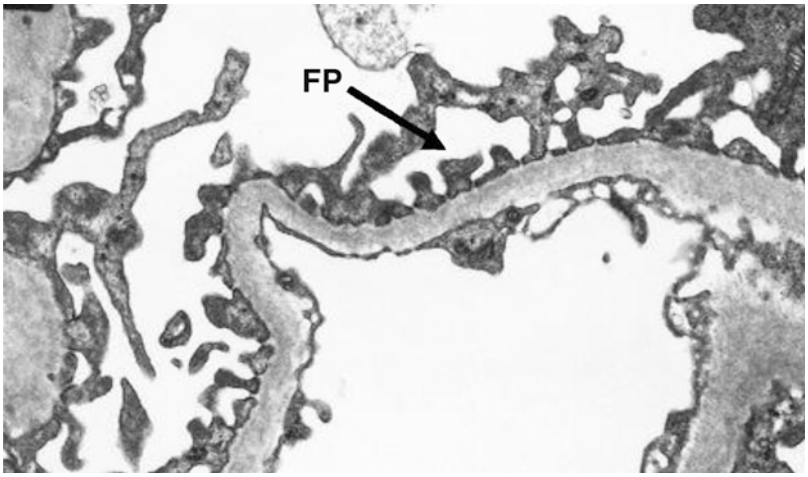
Definition

The clinical hallmarks of immune-mediated kidney injury include the appearance of protein and red blood cells in the urine and, in severe cases, impaired kidney function manifested as a rise in serum creatinine or a fall in the glomerular filtration rate.

Perspective

When the immune system attacks the kidney, most commonly the point of attack is the glomerular compartment. The glomeruli are the filtering units of the kidney and in health only allow passage of small molecules such as waste products, water, glucose, and electrolytes. Circulating



Immune System and Kidney, Fig. 1 The Glomerular filtration barrier. An electron-micrograph of the GBM showing the epithelial side with foot processes (FP), slit diaphragms between the foot processes, the thin endothelial layer on the opposite side of the intervening layer of the extracellular matrix material

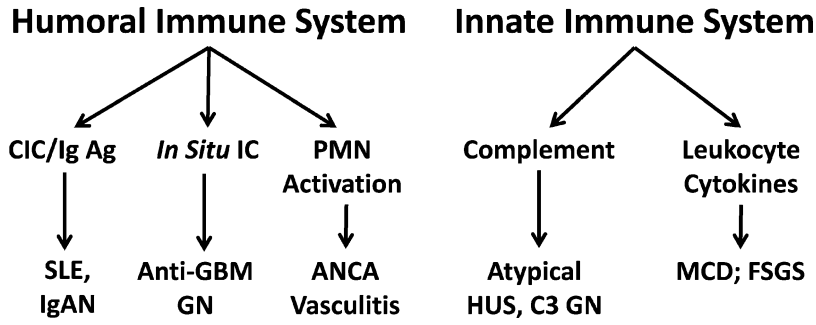
blood cells and serum proteins are largely prevented from crossing the glomerular filtration barrier (GFB). This is made possible because the normal GFB is composed of the glomerular basement membrane (GBM), a unique structure of extracellular matrix material which is sandwiched between a fenestrated endothelial cell layer and the podocytes of the visceral glomerular epithelial cells (Fig. 1). The GFB offers both size and charge selectivity. However, when the GFB is damaged by the immune system, red blood cells, white blood cells, and proteins escape the GFB and pass into the urine. Thus, hematuria and proteinuria are the hallmarks of immune-mediated glomerular injury. In severe cases, hematuria and proteinuria are accompanied by impaired kidney function, clinically manifest as an increased serum creatinine. There are many ways in which the immune system can damage the glomerulus, so hematuria and proteinuria are not specific for any particular type of glomerulonephritis (GN). The precise diagnosis of GN generally requires microscopic examination of kidney tissue, as discussed in the chapter on kidney biopsy (▶ Indications for Biopsy in Autoimmune GN).

The tubulointerstitial compartment of the kidney is also subject to immune-mediated injury. Many of the GNs discussed in this encyclopedia are accompanied by inflammatory or immune damage to the tubulointerstitial space. Chronic damage to the interstitium is associated with chronic kidney disease and predisposes to end-stage kidney failure. Additionally, certain processes not discussed in this section preferentially target the interstitium. An important example is allergic interstitial nephritis. This is essentially an allergic reaction occurring in the kidney's interstitium, often provoked by medications prescribed for other conditions. Allergic interstitial nephritis is important to recognize clinically because it will resolve if the offending agent is removed.

Although multiple components of the immune system are involved in the pathogenesis of most types of GN, these diseases can readily be classified by dominant mechanisms as illustrated in Fig. 2.

Autoantibodies, Immune Complexes, and Glomerulonephritis

Many glomerular diseases are a consequence of autoantibody and immune complex damage. In immune complex diseases, the kidney may be an innocent bystander with circulating immune complexes or immune aggregates initiating damage after depositing in glomeruli. The kidney disease of systemic lupus erythematosus (SLE) represents the prototypical immune complex deposition disease, as discussed in the chapter on lupus nephritis (▶ Lupus Nephritis, Diagnosis and Treatment). Lupus nephritis has several



Immune System and Kidney, Fig. 2 Immune pathways leading to glomerulonephritis. CIC, circulating immune complexes; Ig Ag, Immunoglobulin aggregates; SLE, systemic lupus erythematosus; IgAN, IgA nephropathy; PMN, polymorphonuclear leukocytes; ANCA, anti-neutrophil cytoplasmic antibodies; HUS, hemolytic uremic syndrome; C3 GN, C3 glomerulonephritis; MCD, minimal change disease; FSGS, focal segmental glomerulosclerosis

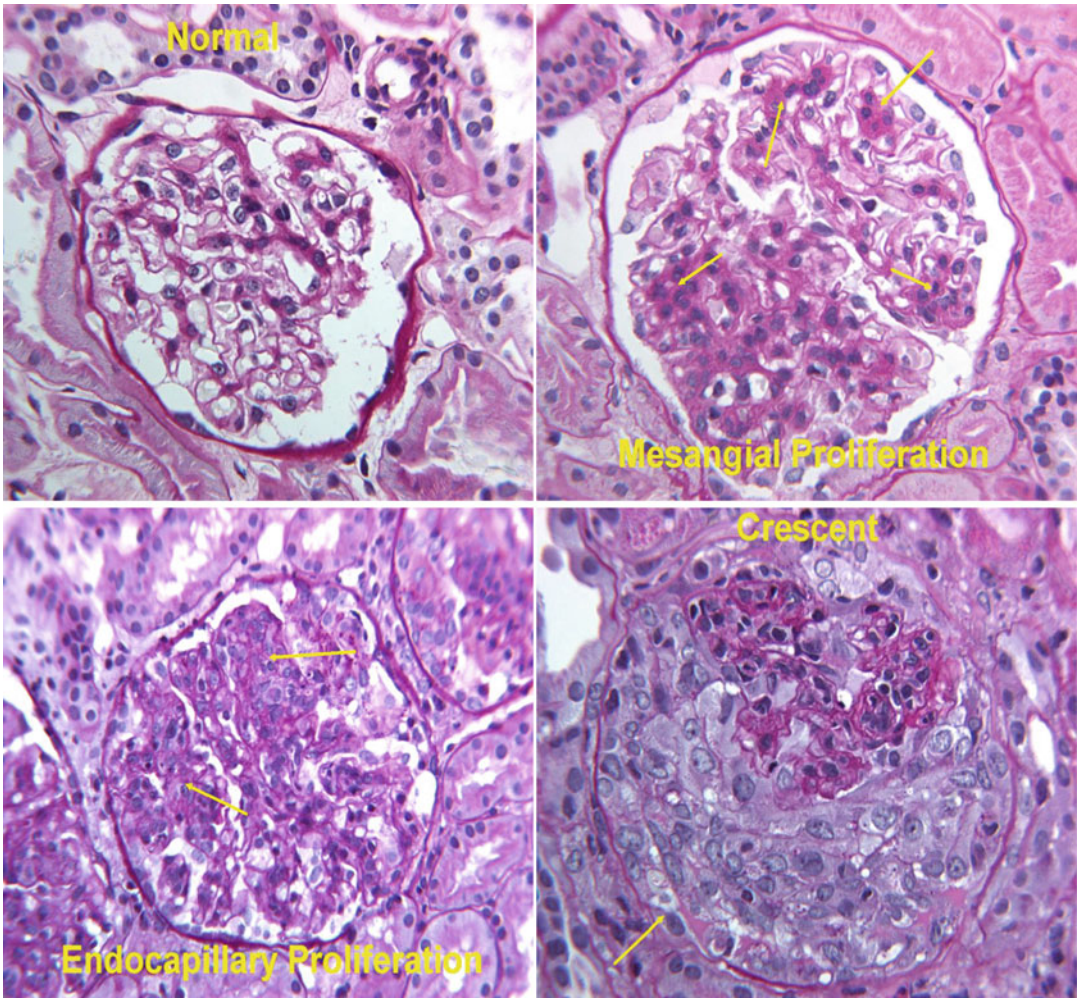
different histologic forms depending on where immune complexes deposit in glomeruli. This is a function of immune complex size, charge, and antigen-antibody affinity. Deposits between the endothelial layer of the GBM and the matrix component (called subendothelial deposits) tend to evoke a very inflammatory response, as they are exposed to circulating immune effector systems such as neutrophils and complement components (Fig. 3). Deposits between the podocytes and the GBM matrix (subepithelial deposits) are somewhat separated from the circulating effectors. These types of deposits do not provoke much inflammation but rather initiate formation of new basement membrane material by intrinsic glomerular cells causing the GBM to become thicker.

In contrast to the pervasive glomerular deposition of immune complexes in lupus nephritis, the immune complex deposition in IgA nephropathy, the most common form of GN in the world, is restricted largely to the glomerular mesangium (Fig. 3). As described in the chapter on IgA nephropathy, these immune aggregates (immune complexes) contain IgA that is antigenic due to aberrant glycosylation of the antibody hinge region (▶ IgA Nephropathy).

Another form of GN in which the immune deposits are restricted to a specific part of the glomerulus is described in the chapter on idiopathic membranous nephropathy. Here, the deposits are subepithelial. In many cases of idiopathic membranous nephropathy the autoantibodies appear to be directed against the podocyte anti-phospholipase A2 receptor. Interestingly, one type of lupus nephritis demonstrates a membranous pattern of immune deposition, but the autoantibodies are not directed against the phospholipase A2 receptor.

In other antibody-mediated GNs, autoantibodies target antigens that are normally expressed by the kidneys. The classic example is anti-GBM disease, in which immune complexes are formed in situ by autoantibodies against exposed neoepitopes in the globular head region of GBM type IV collagen that are usually sequestered. It is not clear how these neoepitopes become exposed. As the chapter on anti-GBM diseases indicates, injury may be restricted to the kidneys or may affect other organs, most commonly the lungs, resulting in a pulmonary hemorrhage-renal syndrome (▶ Anti-Glomerular Basement Membrane Disease).

The theme of antibody-mediated glomerular injury is continued in the chapter on anti-neutrophil cytoplasmic antibody (ANCA)-mediated vasculitis (▶ Vasculitis and the Kidney). These highly inflammatory diseases are often systemic and manifest as pulmonary hemorrhage-renal syndromes. They are characterized by ANCA autoantibodies against neutrophil antigens that activate the neutrophils. But cytokines and the cellular immune system are also actively involved in the pathogenesis of ANCA vasculitis.



Immune System and Kidney, Fig. 3 The glomerular response to mesangial and subendothelial immune complex deposits. A normal glomerulus has an intact Bowman's capsule and open capillary loops. In mesangial immune complex deposition, as may be seen in IgA nephropathy, the *arrows* indicate areas of mesangial proliferation. Endocapillary proliferation, associated with subendothelial immune complex deposition shows no open capillary loops and may be seen in aggressive forms of lupus nephritis. If endocapillary proliferation continues unabated, crescents may form that compress the glomerular capillary network, and breaks of Bowman's capsule (*arrow*) may occur

Thrombotic Microangiopathies

A unique form of glomerular injury, often associated with systemic immunologic disorders is renal thrombotic microangiopathy and is discussed in the chapters covering anti-phospholipid antibody syndrome, scleroderma renal crisis, and TTP/HUS (▶ Scleroderma Renal Crisis). Pathogenic mechanisms include autoantibodies against the endothelium, inability to breakdown von Willebrand factor causing platelet aggregation, and complement dysregulation. These lead to persistent endothelial injury creating an environment that predisposes to microthrombi formation. These microthrombi can obstruct the glomerular capillaries, which causes progressive ischemic kidney injury often associated with hypertension.

The Cellular Immune System

The cellular immune system, possibly through the elaboration of leukocyte cytokines that alter podocyte function and increase glomerular permeability to protein, is thought to play a role in the common childhood GN called minimal change disease (MCD) and the common adult GN known as focal segmental glomerulosclerosis (FSGS) (▶ Spectrum of Minimal Change Disease to Focal Segmental Glomerulosclerosis). However, as pointed out in the chapter on these diseases of the podocyte, mutations of podocyte proteins that affect function of the podocyte filtration barrier are also important and in FSGS, may explain its relative resistance to immunosuppressive therapy.

The Innate Immune System

The innate immune system can impact the kidney in several ways. For many of the antibody-related GNs, complement activation is critical to the pathogenesis of glomerular injury after immune complex deposition. Even in diabetic nephropathy, a major cause of end-stage kidney failure, complement activation, and infiltration of the kidney by immune cells occurs and may be relevant to disease progression (▶ Autoimmunity and Inflammation in Diabetic Nephropathy). Dysregulation of the complement system is also directly involved in certain forms of kidney injury. These defects, either inherited or acquired as described in the chapter on complement, result in uncontrolled complement activation with production of inflammatory products like C5a and the membrane attack complex (▶ Complement Regulation in the Kidney).

Special Circumstances: Kidney Transplantation; Pregnancy

All aspects of the immune system come together in patients who have received a kidney transplant (▶ Impact of Recurrent Autoimmune Diseases in Renal Transplant Outcomes). The chapter on recurrent and relapsing GN in kidney transplant recipients highlights information learned about GN pathogenesis in the setting of pristine glomeruli along with the various forms of immune-mediated organ rejection and how these are addressed clinically.

Pregnancy in patients with immune-mediated kidney diseases is especially challenging. As outlined in the chapter on pregnancy in autoimmune disease, this is an important issue because women during their child-bearing years are often disproportionately affected by autoimmune diseases (▶ Autoimmune Kidney Disease and Pregnancy). Pregnancy may accelerate or activate the underlying autoimmune disease or may accelerate the loss of kidney function in patients with already damaged kidneys from their underlying process. Therapeutic regimens need to be modified to account for fetal well-being. Management of the pregnant patient with autoimmune kidney disease should be undertaken by a multidisciplinary team of physicians, including a high-risk obstetrician, nephrologist, and rheumatologist/immunologist.

Therapy of Immune-Mediated Glomerulonephritis

The therapies of immune-mediated GNs generally involve various combinations of immunosuppressives and corticosteroids (▶ Therapeutic Considerations in Kidney Diseases due to Glomerulonephritis). In some cases, more targeted therapies of specific immune system components have shown promise. Equally important for most forms of proteinuric kidney disease, as discussed in the chapter on kidney protection, control of blood pressure and reduction of proteinuria are crucial ancillary treatments to preserve renal function, especially in cases where immunosuppression does not induce complete remission (▶ Proteinuric Kidney Diseases: Importance of Blood Pressure Control).

In summary, the contributions to this section of the encyclopedia provide a comprehensive picture of how the immune system interacts and injures the kidney, how immune-mediated kidney diseases present clinically, and the therapeutic approaches currently used.

Suggested Reading

Couser WG. Basic and translational concepts of immune-mediated glomerular diseases. J Am Soc Nephrol. 2012;23:381–99.
Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO Clinical Practice Guideline for Glomerulonephritis. Kidney Inter. 2012;(Suppl 2):139–274.
Parikh S, Hebert L, Rovin BH. Protecting the Kidneys in Lupus Nephritis. Int J Clin Rheumatol. 2011;6:529–46.
Tanaka T, Nangaku M. Pathogenesis of tubular interstitial nephritis. Contrib Nephrol. 2011;169:297–310.

Immune-Mediated Mechanisms of the Metabolic Syndrome

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Synonyms

Adipose tissue; Inflammation; Macrophages; Metabolism; Obesity; Type 2 diabetes

Definition

The origins of metabolic syndrome are closely linked to the activation of proinflammatory innate immune pathways. This entry will review the current state of our understanding about how leukocytes contribute to metabolic diseases associated with obesity.

Metainflammation

The obesity epidemic has resulted in a great need to understand the mechanisms that underlie the development of disease that results from metabolic dysfunction. Metabolic syndrome is defined by a constellation of findings that are the result of maladaptive nutrient regulation. These include dyslipidemia, insulin resistance, hypertension, and central obesity that combine to increase the risk for cardiovascular disease and diabetes. While it has long been recognized that inflammatory markers correlate with metabolic syndrome, the mechanistic contribution of this inflammation to the development of insulin resistance and metabolic disease has been exposed only over the last 10–15 years.

This field of research has revealed common pathways at play in the regulation of nutrient metabolism and immune responses. Both systems are built to achieve a similar end, to maintain homeostasis in an organism in the face of a variety of environmental challenges (e.g., fasting or infection). It has become clear over the years that overlap between metabolic regulation and immune responses may be substantial. Unlocking these commonalities creates opportunities to identify ways to block inflammatory activation associated with obesity and thus uncouple obesity from its negative health effects such as cardiovascular disease and diabetes.

The unique nature of the inflammation associated with metabolic disease has led to the coining of the term "metainflammation" to distinguish obesity-associated inflammation from classical inflammatory response mechanisms. Unique characteristics of metainflammation include the chronic nature of the inflammation, the low-grade inflammatory activation observed, the induction by dietary factors and nutrient composition, and the activation of the inflammatory response in metabolic tissues such as adipose tissue that are not typically thought to have a complex immune network.

Overview of Glucose Metabolism

Metabolic diseases such as type 2 diabetes are the result of the breakdown in the normal homeostatic system that maintains nutrient availability (e.g., circulating glucose) within a very narrow range in times of feeding and fasting. Diabetes results from a breakdown in these systems that lead to an increase in blood glucose levels and a dysregulation of how the body generates and

responds to insulin. Type 1 diabetes is an autoimmune disease that targets pancreatic islets and leads to a loss of insulin production and is not associated with obesity. Type 2 diabetes is strongly associated with visceral adiposity and is the result of a failure of peripheral tissues to respond to insulin and lower blood glucose levels.

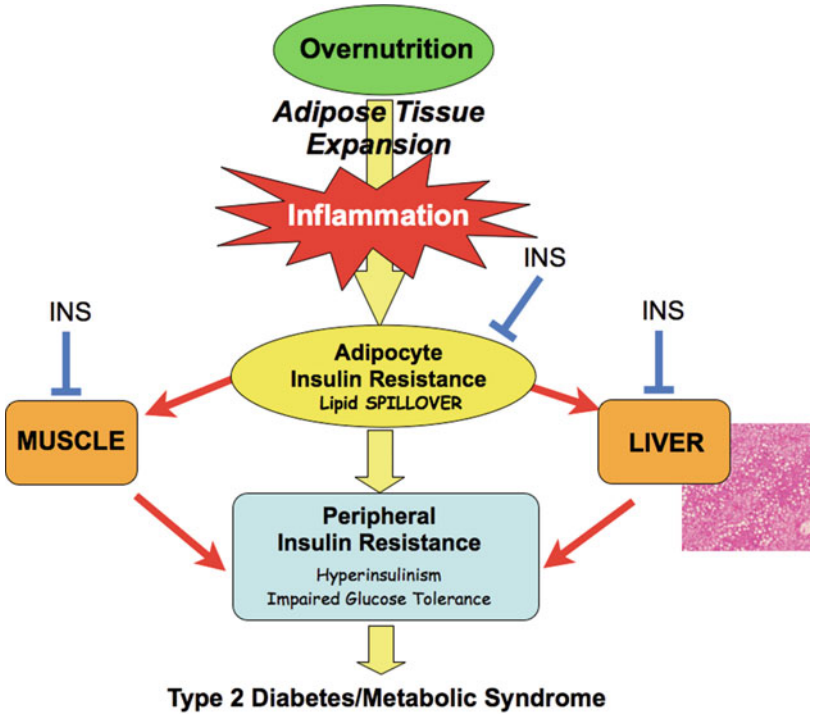
Glucose regulation involves the coordinated function of multiple organs from insulin secreting ß-cells in the pancreas to insulin-responsive tissues that take up glucose from the circulation such as liver, muscle, and adipose tissue. In addition, glucose can be released by the liver, and critical signals from the central nervous system and gastrointestinal system regulate nutrient absorption and utilization. There is evidence that obesity results in proinflammatory activation at multiple control points in this system that combine to limit metabolic flexibility (e.g., how to handle large dietary glucose or fat load).

This review will focus primarily on current understanding of the generation of inflammation in adipose tissue. Adipocytes are the primary energy storage cell in the body that rapidly expand in size and number with nutrient excess. For reasons that are still unclear, adipose tissue hypertrophy is associated with the coordinated activation of inflammatory signals from fat and changes in the function of adipose tissue leukocytes (Fig. 1). The inflammatory signals generated from fat in obesity are diverse and include the production of cytokines such as TNFα, chemokines such as CCL2/MCP1, adipokines such as leptin which can signal to leukocytes, nutrient factors such as saturated free fatty acids, and the suppression of factors with beneficial effects on inflammation such as adiponectin. The net result of adipose tissue inflammation is the impairment of the adipocytes to properly take up, store, and sequester excess nutrients as triglyceride. When this occurs in the face of continued nutrient excess, lipids normally stored in adipose tissue are shunted to other organs not normally designed to handle large lipid loads. Such spillover of lipids contributes to the generation of liver steatosis, lipid droplet accumulation in skeletal muscle, and also the accumulation of lipids in foam cells in atherosclerotic lesions – events that combine to contribute to the morbidities associated with metabolic syndrome. This model has been supported by mouse models which have a profound expansion of adipose tissue in the absence of inflammation that maintains normal glucose homeostasis (Kim et al. 2007b). Overall, this model suggests that by suppressing the inflammatory response to obesity in adipose tissue, adipocyte function can be preserved and the progression towards insulin resistance can be attenuated.

Why is there such overlap between nutrient control and inflammation? Accumulating evidence suggests that many dietary factors associated with obesity can activate the pattern-recognition machinery utilized by the innate immune system to detect pathogens. For example, saturated fatty acids elevated in obesity can bind and activate toll-like receptors TLR4 and TLR2 that lead to NFκB induction (Kim et al. 2007a). Such TLR4 activation can induce the production of intracellular lipids such as ceramides and promote inflammation in multiple tissues (Holland et al. 2011). Fatty acids can also activate the Nod-like receptors and the NLRP3 inflammasome which has been shown to promote insulin resistance through IL-1ß (Vandanmagsar et al. 2011).

Myeloid Cell Contributions to Metabolic Disease

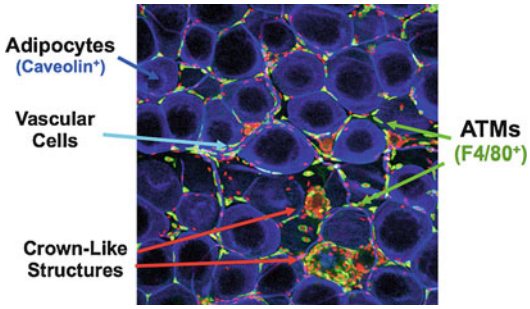
The components of the inflammatory response to obesity were unclear until 2003 when a large population of macrophages were found in adipose tissue in obese patients and rodent models that correlated with inflammation and insulin resistance (Weisberg et al. 2003). This shed light into what is now recognized as an extensive network of leukocytes in adipose tissue that form a large support system of stromal cells for adipocytes (Fig. 2). Adipose tissue macrophages (ATMs) are induced in adipose tissue with obesity and are a main source of inflammatory cytokines such as TNFα. ATM infiltration is more prominent in visceral adipose tissue than



Immune-Mediated Mechanisms of the Metabolic Syndrome, Fig. 1 *Adipose tissue inflammation in the pathogenesis of insulin resistance*. An imbalance between energy uptake and utilization leads to overnutrition. The only tissue designed to store this nutrient excess is adipose tissue which expands via a combination of hypertrophy and hyperplasia. For reasons that are still unclear, this expansion is associated with the triggering of inflammatory signaling cascades as well as an activation of inflammatory leukocytes in fat. Over time, these signals impair the ability of adipocytes to respond to insulin. Such adipocyte insulin resistance leads to increased lipolysis in adipose tissue and the "spillover" of lipids from their normal storage pool in fat to other tissues not normally designed to store lipids. In obesity, tissues such as the liver and skeletal muscle have increased lipid deposition which impairs their ability to properly respond to insulin leading to peripheral insulin resistance seen as impaired glucose tolerance clinically. Over time this puts a stress on the pancreas to produce insulin that can ultimately lead to inadequate insulin production in the fact of insulin resistance which leads to diabetes

in subcutaneous depots, a fact that is important given the associations between visceral adiposity and metabolic syndrome (Aron-Wisnewsky et al. 2009).

An important aspect of ATM biology is the presence of an extensive network of resident macrophages throughout normal adipose tissue development. These resident ATMs (type 2) express surface markers of alternatively activated macrophages (CD206 and CD301) and appear to be dependent upon innate IL-4 production from adipose tissue to provide regulatory functions in fat. With obesity, a distinct ATM population that expresses both F4/80 and CD11c (type 1) are recruited to fat and cluster around dead adipocytes in what are known as crown-like structures (Lumeng et al. 2007a). These ATMs express many classical activation markers and are dependent upon the CCR2/CCL2 chemokine axis for recruitment to adipose tissue in obesity (Weisberg et al. 2005). While these cells express CD11c, it is unclear if these cells have dendritic cell functions. These populational changes in ATMs have led to the model that obesity induces a phenotypic shift in the ATM profile from an M2 to an M1 polarization state. While this is likely an oversimplification of the biology of the system, it has been a useful model from which to frame the problem that has been supported by numerous human and rodent obesity studies.



Immune-Mediated Mechanisms of the Metabolic Syndrome, Fig. 2 *The inflammatory network of adipose tissue*. Confocal microscopy image of visceral adipose tissue from obese mice. C57Bl/6 male mice were fed a high-fat diet (60 % kcal from fat) for 14 weeks prior to imaging. Adipocytes were stained with caveolin (blue). Nuclear stain (PI; red) demonstrates that adipose tissue contains a large number of stromal support cells in direct contact with adipocytes. A dominant population in the fat are F4/80$^+$ adipose tissue macrophages (ATMs; green). These are distributed around adipocytes, along vascular structures, and cluster around dead adipocytes in structures known as crown-like structures

Overall our understanding of the function of ATMs is incomplete. ATMs may play a role in adipose tissue remodeling as experimental ATMs ablation impairs fat angiogenesis and development (Han et al. 2011). There is a coupling between type 1 ATM recruitment and fat cell death. The proinflammatory cytokines made by CD11c$^+$ ATMs contribute to metabolic dysfunction by blocking insulin receptor signaling in adipocytes (Lumeng et al. 2007b). Ablation of CD11c$^+$ ATMs decreased adipose tissue inflammation and led to an improvement in glucose tolerance (Patsouris et al. 2008). Furthermore, there may be a role for adipocytes in the hypoxic response that is associated with fat expansion.

Current models support a model of monocyte trafficking to obese adipose tissue similar to what has been described for atherosclerosis. Obesity induces Ly-6c^{hi} monocytes in the circulation in rodents as well as CD14$^+$CD16$^+$ monocytes in humans (Poitou et al. 2011). Rodent models with attenuation of Ly-6c^{hi} monocytosis have less type 1 CD11c$^+$ ATMs in adipose tissue (Weisberg et al. 2005). This has led to the inference that resident ATMs are derived from Ly-6c^{lo} monocytes, but this has not been directly proven.

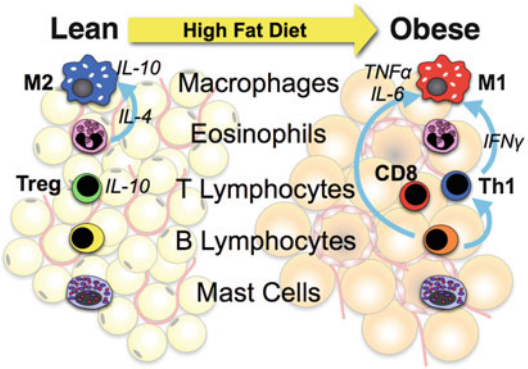
The molecular pathways that contribute to the innate activation of ATMs are varied and include the activation of TLR4, TLR2, and the inflammasome. Obesity induced the activation of NFκB in ATMs specifically which is related to upstream activation signals derived from IKKß as well as noncanonical signaling pathways such as IKKε (Chiang et al. 2009).

Adaptive Immune Responses and Obesity

In mouse models of obesity, ATM induction and activation are most pronounced with prolonged high-fat diet feeding. The possibility that earlier signals derived from adaptive immune responses were suggested by studies investigating the role of adipose tissue lymphocytes in metainflammation (Fig. 3).

CD4$^+$ T cells are found in adipose tissue in smaller quantities than ATMs and undergo similar changes in their profile with diet-induced obesity (Fig. 3). In lean mice and humans, regulatory T cells (Treg) predominate and produce cytokines such as IL-10 which maintain adipocyte insulin sensitivity (Winer et al. 2009). With obesity, fat Tregs decrease in quantity and a population of Th1-skewed conventional T cells (Tconv) are induced (Feuerer et al. 2009). These Tconv secrete IFN-γ which has been shown to contribute to the insulin resistance phenotype induced in obesity (Rocha et al. 2008). The origin of adipose tissue T cells (ATT cells) are not yet established; however, the presence of antigen-specific responses is inferred from evidence that Tconv in fat are clonal and that memory cells are induced with obesity in fat (Yang et al. 2010).

Concurrent with these changes is the induction of adipose tissue CD8$^+$ T cells as well which secrete inflammatory cytokines (Nishimura et al. 2009). This induction appears to be earlier than ATM or CD4$^+$ Tconv in adipose tissue, suggesting that this may be an early initiating event in the cascade of metainflammation. The factors that induce CD8$^+$ cells are not currently known.



Immune-Mediated Mechanisms of the Metabolic Syndrome, Fig. 3 *Leukocyte dynamics in adipose tissue with obesity*. This summarizes the current leukocyte populations identified in adipose tissue and felt to contribute to its function in lean and obese states. Blue lines indicate cross-talk pathways identified between adipose tissue leukocytes to date. Macrophages (ATMs) undergo a populational shift from an M2-dominated state to an M1 state. Eosinophils secrete IL-4 in fat and maintain M2 ATMs in lean mice (Wu et al. 2011). T lymphocytes undergo a populational shift from a Treg dominated to a Th1 and $CD8^+$ T cell-dominated state with obesity. B cells and mast cell also are found in adipose tissue

Finally, recent studies suggest that B cell activation also plays a role in metainflammation (Winer et al. 2011). B cell deficiency was associated with a protection from insulin resistance in rodent models. Evidence suggested that communication between activated B cells and adipose tissue $CD4^+$ T cells may be important for the induction of metainflammation. Surprisingly, an antibody signature was described associated with insulin resistance in humans that further supports a role for adaptive immunity and metainflammation.

Conclusion

To date, almost every leukocyte subtype has been found in adipose tissue, some of which are dynamically changed by obesity, while others are not (Fig. 3). The future challenge is to understand not only how each leukocyte is influenced by nutrient excess but also how these leukocytes communicate and coordinate their responses. Teasing out these connections will take unique approaches given the singular features of metainflammation. Importantly, combining approaches used in the fields of metabolism research and inflammation provides the opportunity to use obesity to tease apart the overlap between metabolic responses and inflammatory signaling. It is the hope that these approaches can benefit the research community while at the same time identifying targets that can be modified to break the link between obesity and disease.

Cross-References

- Atherosclerosis and Cytokines
- Innate Immune Cells in the Liver
- Lymphocytes in Atherosclerosis
- Macrophages, Oxidative Stress, and Atherosclerosis
- NF-κB
- Tregs in the Liver

References

Aron-Wisnewsky J, Tordjman J, Poitou C, Darakhshan F, Hugol D, Basdevant A, Aissat A, Guerre-Millo M, Clement K. Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J Clin Endocrinol Metab. 2009;94:4619–23.

Chiang S-H, Bazuine M, Lumeng CN, Geletka LM, Mowers J, White NM, Ma J-T, Zhou J, Qi N, Westcott D, Delproposto JB, Blackwell TS, Yull FE, Saltiel AR. The protein kinase IKKepsilon regulates energy balance in obese mice. Cell. 2009;138:961–75.

Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, Mathis D. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med. 2009;15:930–9.

Han J, Lee JE, Jin J, Lim JS, Oh N, Kim K, Chang SI, Shibuya M, Kim H, Koh GY. The spatiotemporal development of adipose tissue. Development. 2011;138:5027–37.

Holland WL, Bikman BT, Wang LP, Yuguang G, Sargent KM, Bulchand S, Knotts TA, Shui G, Clegg DJ, Wenk MR, Pagliassotti MJ, Scherer PE, Summers SA. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid–induced ceramide biosynthesis in mice. J Clin Invest. 2011;121(5):1858–70. doi:10.1172/JCI43378.

Kim F, Pham M, Luttrell I, Bannerman DD, Tupper J, Thaler J, Hawn TR, Raines EW, Schwartz MW. Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. Circ Res. 2007a;100:1589–96.

Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, Jelicks LA, Mehler MF, Hui DY, Deshaies Y, Shulman GI, Schwartz GJ, Scherer PE. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest. 2007b;117:2621–37.

Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest. 2007a;117:175–84.

Lumeng CN, Deyoung SM, Saltiel AR. Macrophages block insulin action in adipocytes by altering expression of signaling and glucose transport proteins. Am J Physiol Endocrinol Metab. 2007b;292:E166–74.

Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med. 2009;15:914–20.

Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab. 2008;8:301–9.

Poitou C, Dalmas E, Renovato M, Benhamo V, Hajduch F, Abdennour M, Kahn JF, Veyrie N, Rizkalla S, Fridman H, Sautes-Fridman C, Clement K, Cremer I. CD14dimCD16+ And CD14+CD16+ monocytes in obesity and during weight loss: relationships with Fat mass and subclinical atherosclerosis. Atertio Thromb Vasc Biol. 2011;31(10):2322–30.

Rocha VZ, Folco EJ, Sukhova G, Shimizu K, Gotsman I, Vernon AH, Libby P. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. Circ Res. 2008;103:467–76.

Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med. 2011;17:179–88.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112:1796–808.

Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, Charo I, Leibel RL, Ferrante AW. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest. 2005;116:115–24.

Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y, Drucker D, Endleman E, Winer D, Dosch HM. Normalization of obesity-associated insulin resistance through immunotherapy: CD4+ T cells control glucose homeostasis. Nat Med. 2009;15:921–9.

Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, Leong HX, Glassford A, Caimol M, Kenkel JA, Tedder TF, McLaughlin T, Miklos DB, Dosch HM, Engleman EG. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med. 2011;17:610–7.

Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan H, Bando JK, Chawla A, Locksley RM. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science. 2011;332(6026):243–7.

Yang H, Youm YH, Vandanmagsar B, Ravussin A, Gimble JM, Greenway F, Stephens JM, Mynatt RL, Dixit VD. Obesity increases the production of proinflammatory mediators from adipose tissue T cells and compromises TCR repertoire diversity: implications for systemic inflammation and insulin resistance. J Immunol. 2010;185:1836–45.

Immunobiology of Interleukin-21

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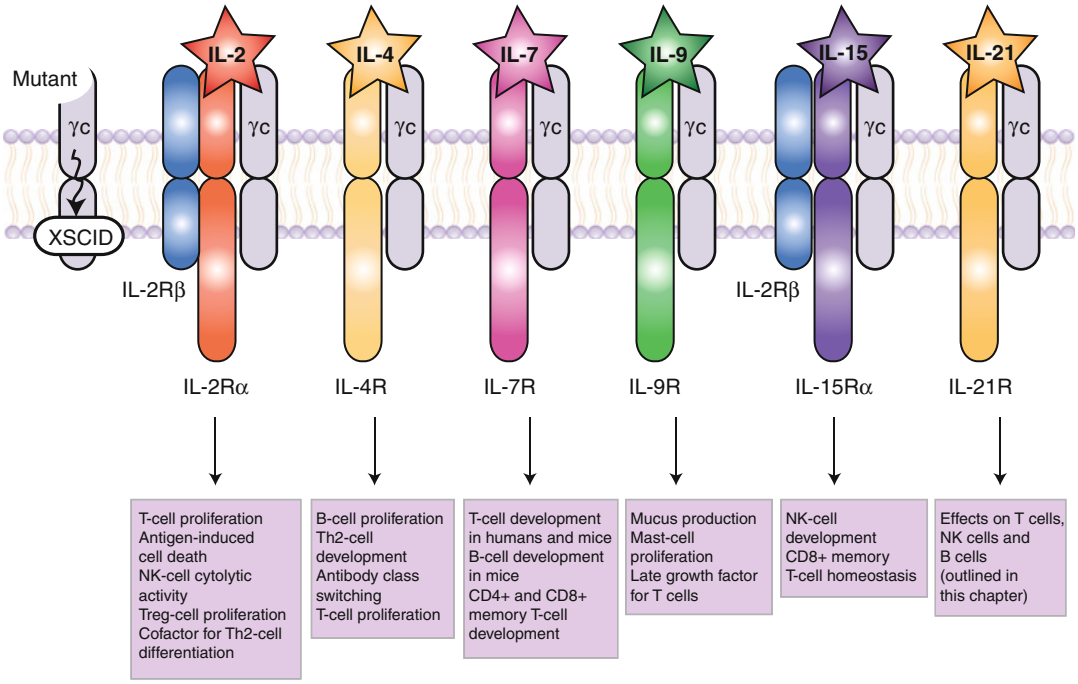
Synonyms

IL-21; Za11

Definition

Interleukin-21 (IL-21) is a cytokine produced by activated $CD4^+$ T cells and NK T cells

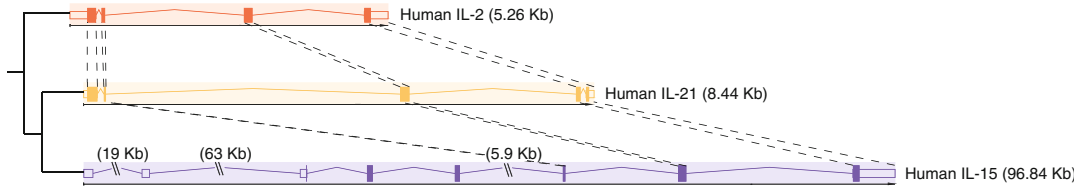


Immunobiology of Interleukin-21, Fig. 1 The common gamma-chain (gc) cytokine receptor family. The IL-21 receptor is a member of a family of receptors that share gc. In addition to gc, each of these receptors has one or more distinctive receptor components. Mutations in gc result in X-linked severe combined immunodeficiency (XSCID); the severity of this disease results from defective signaling through all these receptors (Figure adapted from (Spolski and Leonard 2008))

(Parrish-Novak et al. 2000; Coquet et al. 2007). The IL-21 receptor (IL-21R) is a heterodimeric receptor, which consists of an IL-21-specific IL-21R α-chain and the common γ-chain (CD132) (Asao et al. 2001). Since its discovery over a decade ago, a wide body of literature has provided evidence that IL-21 plays an important role in innate and adaptive immune responses (Leonard and Spolski 2005). This entry focuses on the role of IL-21 in both humoral and cell-mediated adaptive immune responses directed against foreign and self-antigens.

Discovery

In 2000, Ozaki et al. scanned genomic sequences for "virtual" open reading frames (ORFs) and cloned a novel interleukin receptor related to the IL-2Rβ chain. They identified a type 1 cytokine receptor containing typical features, including four conserved Cys residues in the putative extracellular region and the highly conserved Trp-Ser-X-Trp-Ser (WSXWS) cytokine-binding domain (Ozaki et al. 2000). In a parallel study, Parrish-Novak et al. identified an expression tag containing a signal peptide and predicted amphipathic helix as a newly found class 1 cytokine receptor and designated it IL-21R (Parrish-Novak et al. 2000). This gene was also thought to be a signaling subunit, because the intracellular domain included the classic Box 1 and Box 2 motifs, which are important in the signal transduction that is initiated following ligation of cytokine receptors. These characteristics are common to the type 1 cytokine receptor superfamily, which utilize the common γ-chain (γc) as part of the receptor complex as shown in Fig. 1. Located on human chromosome 16p11 downstream of IL-4Rα, the full-length cDNA sequence encodes a 538 amino acid cytokine receptor. IL-21R shows highest identity to IL-2Rβ and IL-4Rα.



Immunobiology of Interleukin-21, Fig. 2 Comparison of the structures of the human IL-21, IL-2, and IL-15 genes. Exons are depicted as *boxes*. Coding regions are *shaded*, and untranslated regions are *open*. The loci are drawn to scale (from Ensembl Genome Browser schematics) except for the large introns in *IL-15*, with the size of each omitted intron as indicated. The tree diagram at the *left* indicates relatedness as measured by alignment of the mature polypeptide sequences (Clustal method, Lasergene software package, DNASTAR) (Figure adapted from (Parrish-Novak et al. 2002))

Using a functional cloning approach, Parrish-Novak et al. identified a ligand for IL-21R, designating it IL-21 (Parrish-Novak et al. 2000). The human *Il21* gene encoded a fully processed mature protein of 133 amino acids (~15,000 MW) and is located on the human chromosome 4q26–27. IL-21 consisted of a four-helix bundle cytokine domain and showed a close relationship to other class I cytokines that also bind the common γ-chain, specifically IL-2 (13.7 % identity) and IL-15 (20 % identity), which have the highest structural and primary sequence similarity to IL-21 (Parrish-Novak et al. 2000) as shown in Fig. 2.

Biological Source of IL-21

IL-21 is produced by $CD4^+$ T cells and NK T cells (Parrish-Novak et al. 2000; Coquet et al. 2007). Once activated, in response to antigen peptide presented in the context of MHC class II molecules on the surface of antigen-presenting cells, $CD4^+$ T cells differentiate into distinct subsets of T helper (Th) cells (e.g., Th_1, Th_2, Th_9, Th_{17}, Tfh). Th cells assist in diverse aspects of adaptive immune responses promoting anti-pathogen activities. NK T cells, in turn, are innate-like T lymphocytes that recognize glycolipid antigens in the context of the CD1 molecule and are thought to influence the adaptive immune response due to their ability to rapidly release cytokines. Interestingly, it has been shown that IL-21 can regulate its own production, in a STAT3-dependent manner (Caprioli et al. 2008). Thus IL-21 production through autocrine amplification can aid $CD4^+$ T cell proliferation and survival (Nurieva et al. 2008; Vogelzang et al. 2008).

Multiple T helper cell subsets have been reported to express IL-21, including T follicular helper (Tfh), Th_{17}, and Th_2 cells (Chtanova et al. 2004; Vogelzang and King 2008), but the Tfh cell subset has been shown to produce the greatest amounts of IL-21 (Chtanova et al. 2004). T_{fh} cells are found in the B cell follicles of secondary lymphoid organs and are important in providing help for the processes of somatic hypermutation and affinity maturation of antibody in B cells during the germinal center (GC) reaction (King 2009). High production of IL-21 by Tfh cells contributes to the T cell help delivered to B cells, as discussed below. Th_2 cells produce cytokines such as IL-4, and Th_2 effector cells are associated with the promotion of IgE and eosinophilic responses against extracellular bacteria, parasites, and toxins in non-lymphoid tissues. By contrast Th_1 cells produce the canonical cytokine IFNγ, assisting macrophages and B cells to clear intracellular pathogens. Th_{17} cells, in turn, produce IL-17, which is involved in the activation, recruitment, and migration of neutrophils, and as such, Th_{17} cells are critical for host defense against extracellular bacteria and fungi. The linear delineation of Th subsets has been recently challenged by information gained from epigenetic studies that provide evidence that Th subsets retain a considerable degree of plasticity (Wei et al. 2009). Plasticity in Th cells that were previously thought to have undergone irreversible lineage commitment emphasizes the

importance of local cellular interactions and other microenvironmental cues in establishing the diversity of Th cell phenotype and function.

IL-21:IL-21R Signaling

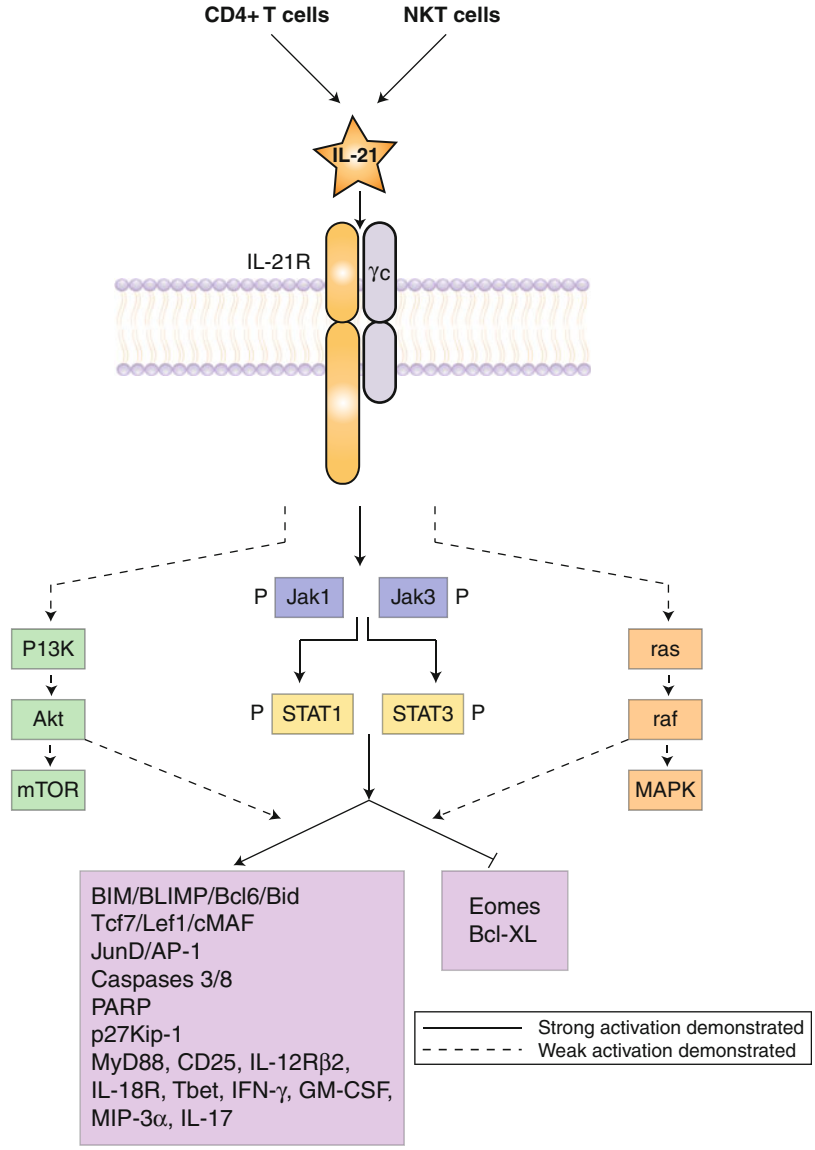
In a series of experiments designed to test the biological properties of the novel cytokine, IL-21 was found to induce proliferative responses in the BaF3, pre-B cell line expressing IL-21R (Parrish-Novak et al. 2000). BaF3 cells, constructed to stably express full-length IL-21R, only initiated a cellular proliferation signal when in contact with its corresponding ligand, IL-21. Conditioned medium from more than 100 primary cells and immortalized cell lines was tested for activity and medium from phorbol myristate acetate (PMA)-/ionomycin-activated human peripheral $CD3^+$ T cells induced proliferation of BaF3/IL-21R cells, but not wild-type BaF3 cells.

IL-21R is expressed on a wide variety of immune cells including B cells, $CD4^+$ and $CD8^+$ T cells, NK cells, dendritic cells, macrophages, and nonimmune cells such as keratinocytes (Ozaki et al. 2000; Parrish-Novak et al. 2000), highlighting the vast influence IL-21 has over a range of cell types. IL-21 acts through signal transduction of its unique receptor chain, IL-21R, in association with (γc), similarly utilized by IL-2, IL-4, IL-7, IL-9, and IL-15, defining it as a member of the IL-2 family of cytokines (Leonard and Spolski 2005). The IL-21R forms a heterodimeric receptor complex with the common gamma-chain and cell lines that lack γc are deficient in IL-21 induced signaling, which can be restored upon reconstitution of γc (Asao et al. 2001). As shown in Fig. 3, binding of IL-21 activates the Janus kinases (JAK)/signal transducer and activator of transcription (STAT) pathway, signaling primarily through STAT1 and STAT3 (Asao et al. 2001; Zeng et al.). The cytoplasmic domain of IL-21R has a membrane-proximal Box 1 motif and six tyrosine residues, implicated as Janus kinase (JAK)-binding sites. The heterodimer IL-21R complex, like IL-15, stimulates activation of JAK1 and JAK3 (Asao et al. 2001). IL-21 has a direct association with JAK 1 since it has been coprecipitated with IL-21R in PHA-activated peripheral blood mononuclear cells (Ozaki et al. 2000). IL-21 activation of JAK3/STAT3 is likely to explain the ability IL-21 to support the survival of both T and B cells during immune responses. STAT3 provides an important counterbalance to STAT5 during the differentiation of T helper cell subsets (Yang et al. 2011). Additionally, phosphatidylinositol-3-kinase (PI3K)-AKT and mitogen-activated protein kinase (MAPK) signaling pathways have been reported to act downstream of IL-21 signaling to influence lymphocyte survival and fate decisions (Zeng et al. 2007).

Role of IL-21 in $CD4^+$ T Cell Immune Responses

Cytokines have an important regulatory role for the homeostasis of hematopoietic progenitor cells (HPCs). HPCs reside in the bone marrow where they undergo proliferation and differentiation to become mature leukocytes and erythrocytes. Upon maturation, leukocytes and erythrocytes are released into the blood to carry out their respective functions. A recent study has described a crucial role for IL-21 in regulating the homeostasis of HPCs (Kaplan et al. 2011). This finding supports earlier observations that mice transgenic for overexpression of IL-21 displayed an increase in hematopoiesis (Ozaki et al. 2006). However, thymic development is normal in mice deficient in IL-21 signaling, suggesting that IL-21 does not play a major role in T cell development or selection (Kasaian et al. 2002; Ozaki et al. 2004). Conversely, evidence supports a role for IL-21 in T cell priming and can costimulate signals delivered through the T cell receptor (TCR). While antigen naïve $CD4^+$ and $CD8^+$ T cells express low levels of IL-21R mRNA, this is markedly upregulated upon T cell receptor (TCR) stimulation (King et al. 2004; Wu et al. 2005).

IL-21 has the ability to influence the differentiation of multiple T helper subsets, including both Th_1 and Th_2 responses. These somewhat diverse outcomes may be attributed to the



Immunobiology of Interleukin-21, Fig. 3 IL-21R signaling pathway. IL-21 signals via the Janus-activated kinase (JAK)/STAT pathway with STAT1 and STAT3 as primary targets. Signaling through the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways may also be relevant in certain physiologic or pathologic conditions (Figure adapted from (Davis et al. 2007))

combined effect of IL-21 with other cytokines and signals present in the microenvironment during antigen priming and a broad role for IL-21 in lymphocyte survival as well as differences in the behavior of immune cells in response to IL-21 between in vitro and in vivo conditions (Agnello et al. 2003; Strengell et al. 2004; Pesce et al. 2006; Frohlich et al. 2007).

IL-21 has also been shown to support the generation and/or survival of IL-17A producing (Th_{17}) proinflammatory T helper subset. In vitro studies demonstrated enhanced production of IL-17 in the presence of IL-21, and an ability of IL-21 to substitute for IL-6 in Th_{17} differentiation, which may reflect the ability of both cytokines to activate STAT3. A series of studies indicate a link between IL-21 and the induction and expansion of Th_{17} cells and disease severity in a murine autoimmune model of brain inflammation, experimental autoimmune encephalomyelitis (EAE) (Vogelzang and King 2008). The findings demonstrated a critical role for IL-21 in Th_{17} generation when the key differentiation factor IL-6 was unavailable. By contrast, in the

presence of abundant levels of IL-6, IL-21 was dispensable: *Il21r*$^{-/-}$ mice display normal susceptibility to EAE, shown first by Sonderegger et al. and later confirmed by other groups (Vogelzang and King 2008).

The role IL-21 plays in autoimmune disease may be attributed to its known role in regulating the survival and differentiation of T cells and the generation of antibody-forming B cells (Nurieva et al. 2008; Vogelzang et al. 2008). As discussed below, IL-21 has an important role in the survival and/or differentiation of B cells during the GC reaction. In addition, the autocrine effect of IL-21 on Tfh cell expansion and survival influences the magnitude and output of the GC reaction. GC reactions in models of autoimmunity (Luzina et al. 2001; Vinuesa et al. 2009) may occur in ectopic tertiary lymphoid structures, which have been suggested to provide a site for the generation of self-reactive autoantibodies (Kendall et al. 2007). Interestingly, a novel systemic lupus erythematosus (SLE)-like autoimmune syndrome described in Sanroque mice associates elevated levels of IL-21 with spontaneous germinal center formation and autoimmune pathology (Vinuesa et al. 2005). However, studies from *Il21r*$^{-/-}$ mice indicate that while IL-21 may under normal conditions act to support T_{FH} cells, IL-21-IL-21R interactions are redundant for the generation of T_{FH} cells and GC formation. Similar to Th_{17} generation, other STAT3 signaling cytokines such as IL-6 can act in place of IL-21 for T_{FH} cell generation and survival (Eto et al. 2011). Taken together, studies suggest a contributing role for IL-21 in Tfh cell survival and the persistence of a GC, which is essential for the time-consuming process of somatic hypermutation (SHM) and production of affinity-matured antibodies.

T regulatory cells (Tregs) are critically dependent on IL-2 for their suppressive function and survival. Given that FoxP3 expression is upregulated upon IL-2 signaling through STAT5a/b, it is interesting to find that FoxP3 expression is enhanced in IL-21-deficient mice and IL-21 downregulates FoxP3 expression and inhibits Treg suppressor function (Nurieva et al. 2007). IL-21 has been shown to inhibit TGFβ-driven differentiation of naïve Th cells into Foxp3$^+$ Tregs (Fantini et al. 2007). Additionally, IL-21 was found to be highly effective at overcoming effective at overcoming Treg-mediated suppression of effector T cells by acting directly on the effector population rather than the Tregs (Peluso et al. 2007).

Role of IL-21 in CD8$^+$ T Cell Immune Responses

IL-21 has a profound effect on the expansion of CD8$^+$ T cells (Allard et al. 2007), and while IL-21 is likely to affect both their proliferation and survival, the mechanism by which IL-21 imparts its effects on lymphocytes remains incompletely understood (Vogelzang and King 2008). CD8$^+$ T cell responses to chronic viral infection are sustained in the presence of IL-21. A number of studies have demonstrated a role for IL-21 in supporting chronic, but not acute, responses to viral infections by preventing CD8$^+$ T cell exhaustion (Elsaesser et al. 2009; Frohlich et al. 2009; Yi et al. 2009).

Transgenic overexpression of IL-21 resulted in a predominantly expanded memory-phenotype CD8$^+$ T cell population, as well as increased number of memory-phenotype CD4$^+$ T cells, but, surprisingly, had little effect on B cell numbers (Allard et al. 2007). The expansion of memory-phenotype CD8$^+$ T cells in the presence of excessive IL-21 is likely to reflect both enhanced proliferation and survival. However, CD8$^+$ T cell development appears normal in *Il21r*$^{-/-}$ mice suggesting that while IL-21 can provide a strong survival signal, other factors compensate under homeostatic conditions (Kasaian et al. 2002). Given the essential role of CD8$^+$ T cells in type 1 diabetes (T1D) pathogenesis, it is interesting to note that CD8$^+$ T cells show increased proliferation in NOD mice in response to elevated levels of IL-21 (King et al. 2004; McGuire et al. 2011a, b) and that IL-21 responsiveness by CD8$^+$ T cells was critical for rejection of islet allografts (McGuire et al. 2011b).

In addition to the demonstrated role of IL-21 to costimulate the proliferation of T cells stimulated through the TCR, IL-21 has been shown to

enhance IFNγ production and cytotoxic function of $CD8^+$ effector T cells (Kasaian et al. 2002). IL-21 can also stimulate proliferation and acquisition of cytotoxic effector functions of $CD8^+$ T cells in conjunction with IL-15 and IL-7 (and perhaps IL-2), in both in vitro and in vivo settings (Zeng et al. 2005). The ability of IL-21 to synergize cytokine-mediated proliferation of $CD8^+$ T cells occurs in the absence of overt TCR signaling which suggests that IL-21 may play a cooperative role in antigen-independent expansion. However, these findings have not yet been reproduced with MHC class $I^{-/-}$ cells. Additionally, the activation status of a $CD8^+$ T cell influences its response to IL-21. Combined cytokine effects are more efficient in $CD8^+$ memory T cells; however, these cells are less responsive to IL-21 costimulating antigen dependent proliferation, compared to naïve $CD8^+$ T cells (Liu et al. 2007; Cui et al. 2011).

Role of IL-21 in B Cell Immune Responses

IL-21R is expressed on both immature and mature B cells and further upregulated upon TLR ligand stimulation or antigen binding the B cell receptor (BCR). The seminal paper showing a role for IL-21 in antibody responses showed a significant decrease in IgG1 production in *Il21r*$^{-/-}$ mice following immunization (Ozaki et al. 2002). Mice lacking both IL-4 and IL-21R exhibited a significantly more pronounced phenotype, with dysgammaglobulinemia, characterized by a severely impaired IgG response (Ozaki et al. 2002). The demonstration that IgG production in response to T-dependent antigen is severely compromised in *Il21r*$^{-/-}$*Il4*$^{-/-}$ mice underscores the cooperative nature of IL-21 and IL-4 during the generation of antibody-forming cells (Ozaki et al. 2002). An interesting observation in *Il21r*$^{-/-}$ mice is an increase in the amount of IgE detected in serum following immunization (Ozaki et al. 2002), the dominant isotype produced in allergy and atopy.

Studies utilizing *Il21r*$^{-/-}$ mice have consistently demonstrated that IL-21 has an early role in the survival or differentiation of antibody-forming cells during the GC reaction. However, whether IL-21 predominantly affects the generation of GC B cells, plasma cells, or memory B cells remains controversial. A nonredundant role for IL-21 in the generation of GC and plasma B cells has been demonstrated (King et al. 2010; Linterman et al. 2010; Zotos et al. 2010), whereas other studies have demonstrated that GC B cells do develop and plasma cells can be found in the bone marrow of *Il21r*$^{-/-}$ mice after immunization or infection (Rankin et al. 2011). In addition TLR7 ligation can overcome the requirement for IL-21R signaling in B cells for antibody production following virus infection (Bessa et al. 2010; Rankin et al. 2011). Transgenic expression of IL-21 has been shown to increase the number of IgG1 class-switched B cells and upregulate the germinal centre B cell transcription factor Bcl6 (Ozaki et al. 2004). Excessive levels of IL-21 also lead to preferential differentiation of B cells into plasma cells and Blimp-1 upregulation (Ozaki et al. 2004).

IL-21 has been shown to either assist or hinder B cell responses in vitro, depending on the accompanying activation signals. When paired with the ligation of CD40 in vitro, IL-21 has been shown to increase human B cell proliferation (Parrish-Novak et al. 2000; Good et al. 2006). Conversely, when IL-21 was combined with BCR stimulation or TLR ligands in vitro, B cell responses were reduced. Furthermore, proliferation is inhibited and apoptosis is induced when IL-21 is present in a response against TLR ligands (Leonard and Spolski 2005; Good et al. 2006). Similar to the inconsistent findings on the role of IL-21 in T helper cell differentiation, some of these disparate findings for the effects of IL-21 on B cell behavior may be attributed to the source of antigenic stimulation and the potential for other factors to compensate for IL-21 in vivo.

IL-21 in Chronic Inflammation and Autoimmune Disease

The *Il2*/*Il21* genetic region on chromosome 3 in mice and chromosome 4 in humans has been

identified through genome-wide association studies to be associated with several autoimmune and inflammatory diseases (Hill et al. 2008; Maiti et al. 2010). An association between T1D incidence and the human chromosome 4q27 was initially described, with a subsequent independent case–control cohort confirming this association with patients with T1D and rheumatoid arthritis. Additional autoimmune and inflammatory diseases revealing associations with the *Il2*/*Il21* region include multiple sclerosis, celiac disease, ulcerative colitis, autoimmune thyroid disease (Graves' disease), and systemic sclerosis (Zhernakova et al. 2009). Recent studies have confirmed that the genetic association with lupus within the *Il2*/*Il21* linkage disequilibrium block is localized to IL-21 (Hughes et al. 2011). The gene for IL-21 has also been associated with human T1D (additively to associations with *Il21r*) and other autoimmune diseases including celiac disease, SLE, and a proposed association with multiple sclerosis (Monteleone et al. 2009).

In light of the evidence that IL-21 is redundant for many immune responses, the prominent role of IL-21 in autoimmune diseases remains unexplained. One possibility is that IL-21 can become the dominant voice in autoimmune lesions within non-lymphoid tissue that lay outside the growth factor-rich environment of lymphoid tissue. In this context, IL-21 is known to play an important role in chronic inflammatory lesions (Leonard and Spolski 2005), such as in the pancreas of NOD mice, where responses to elevated levels of IL-21 are associated with spontaneous expansion of T cells, suggestive of ongoing autoreactivity (King et al. 2004). The critical link of T1D disease progression in NOD mice to responsiveness to IL-21 was demonstrated with *Il21r*$^{-/-}$ mice (Datta and Sarvetnick 2008; Sutherland et al. 2009), which were completely protected from disease. A subset of CD4^{+} T cells has been recently described in pancreatic infiltrates of diabetic NOD mice that express the gut-homing chemokine CCR9 and are the major source of IL-21 in the islet lesion (McGuire et al. 2011a). These IL-21-producing CCR9^{+} Th cells were also observed in human lymphoid tissue and were elevated in the peripheral blood of most Sjögren's syndrome patients (McGuire et al. 2011a). The observation of IL-21-producing CCR9^{+} Th cells in the inflamed lesions of both the salivary glands and pancreas suggested a role for IL-21-producing Th cells in directing the regional specification of autoimmune diseases.

Similarly, IL-21 is important in systemic autoimmune diseases; in the BXSB-Yaa mouse strain (Ozaki et al. 2004), a model of lupus neutralizing circulating IL-21 with an IL-21 receptor trap (IL-21R/Fc) delayed disease progression, increasing survival and lowering levels of serum anti-DNA antibodies. Treatment with IL-21R/Fc has effectively reduced disease in numerous murine models of autoimmune disease, including lupus, T1D, EAE, and RA (both collagen-induced arthritis and adjuvant-induced arthritis) (Vogelzang and King 2008; McGuire et al. 2011a).

Following the initial observation of increased IL-21 mRNA in type 1 diabetes-prone NOD mice (King et al. 2004), elevated levels of IL-21 have also been observed in association with human and murine autoimmune diseases and chronic inflammation including rheumatoid arthritis (RA), Crohn's disease, SLE, Sjögren's syndrome (Wang et al. 2007; Vogelzang and King 2008), and autoimmune uveitis (Liu et al. 2009). Elevated IL-21 has also been demonstrated in human inflammatory diseases such as celiac disease, in biopsies of ulcerative colitis patients (Yamamoto-Furusho et al. 2010), and in Crohn's disease, as well as in helicobacter pylori infections, further strengthening the suggestion that elevated levels of IL-21 may play a role in inflammation irrespective of the etiology.

Summary

IL-21 is a pleiotrophic cytokine with an important role in the regulation of the immune system. While the immune system is viewed globally, cytokines generally impart their effects locally – across short distances between cells. The production of IL-21 by CD4^{+} T cells and

the expression of IL-21R on a variety of immune cells indicate that IL-21 is a T helper cytokine that is poised to influence the development and magnitude of immune responses. IL-21 signals through multiple pathways including STAT3/Jak3 and PI3K and is therefore capable of affecting immune cells whose survival and differentiation is influenced by the activation of these pathways, such as B cells and both $CD4^+$ and $CD8^+$ T cells. The contribution of IL-21 to the immune response is influenced by the presence of other cytokine growth factors in the microenvironment that can offer overlapping or synergistic signals to affect cell fate decisions. The observed redundancy for IL-21 during immune responses within secondary lymphoid tissue contrasts with the more prominent influence reported for IL-21 during chronic inflammation within non-lymphoid tissues.

The strong association of elevated amounts of IL-21 with autoimmune and chronic inflammatory disease has led to a considerable number of studies demonstrating that blockade of IL-21-IL-21R interactions can inhibit the development of disease in animal models. The autoimmune and inflammatory diseases shown to be under the control of IL-21 include both organ-specific and systemic diseases that are T cell mediated and B cell mediated. IL-21 remains a promising therapeutic target for treatment of human autoimmune and chronic inflammatory disease as we await the results of ongoing clinical trials.

References

Agnello D, Lankford CS, et al. Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. J Clin Immunol. 2003;23(3):147–61.

Allard EL, Hardy MP, et al. Overexpression of IL-21 promotes massive CD8+ memory T cell accumulation. Eur J Immunol. 2007;37(11):3069–77.

Asao H, Okuyama C, et al. Cutting edge: the common gamma-chain is an indispensable subunit of the IL-21 receptor complex. J Immunol. 2001;167(1):1–5.

Bessa J, Kopf M, et al. Cutting edge: IL-21 and TLR signaling regulate germinal center responses in a B cell-intrinsic manner. J Immunol. 2010;184(9):4615–9.

Caprioli F, Sarra M, et al. Autocrine regulation of IL-21 production in human T lymphocytes. J Immunol. 2008;180(3):1800–7.

Chtanova T, Tangye SG, et al. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. J Immunol. 2004;173(1):68–78.

Coquet JM, Kyparissoudis K, et al. IL-21 is produced by NKT cells and modulates NKT cell activation and cytokine production. J Immunol. 2007;178(5):2827–34.

Cui W, Liu Y, et al. An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells. Immunity. 2011;35(5):792–805.

Datta S, Sarvetnick NE. IL-21 limits peripheral lymphocyte numbers through T cell homeostatic mechanisms. PLoS One. 2008;3(9):e3118.

Davis ID, Skak K, et al. Interleukin-21 signaling: functions in cancer and autoimmunity. Clin Cancer Res. 2007;13(23):6926–32.

Elsaesser H, Sauer K, et al. IL-21 is required to control chronic viral infection. Science. 2009;324(5934):1569–72.

Eto D, Lao C, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. PLoS One. 2011;6(3):e17739.

Fantini MC, Rizzo A, et al. IL-21 regulates experimental colitis by modulating the balance between Treg and Th17 cells. Eur J Immunol. 2007;37(11):3155–63.

Frohlich A, Marsland BJ, et al. IL-21 receptor signaling is integral to the development of Th2 effector responses in vivo. Blood. 2007;109(5):2023–31.

Frohlich A, Kisielow J, et al. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science. 2009;324(5934):1576–80.

Good KL, Bryant VL, et al. Kinetics of human B cell behavior and amplification of proliferative responses following stimulation with IL-21. J Immunol. 2006;177(8):5236–47.

Hill NJ, King C, et al. Recent acquisitions on the genetic basis of autoimmune disease. Front Biosci. 2008;13:4838–51.

Hughes T, Kim-Howard X, et al. Fine-mapping and transethnic genotyping establish IL2/IL21 genetic association with lupus and localize this genetic effect to IL21. Arthritis Rheum. 2011;63(6):1689–97.

Kaplan MH, Glosson NL, et al. STAT3-dependent IL-21 production from T helper cells regulates hematopoietic progenitor cell homeostasis. Blood. 2011;117(23):6198–201.

Kasaian MT, Whitters MJ, et al. IL-21 limits NK cell responses and promotes antigen-specific T cell activation: a mediator of the transition from innate to adaptive immunity. Immunity. 2002;16(4):559–69.

Kendall PL, Yu G, et al. Tertiary lymphoid structures in the pancreas promote selection of B lymphocytes in autoimmune diabetes. J Immunol. 2007;178(9):5643–51.

King C. New insights into the differentiation and function of T follicular helper cells. Nat Rev Immunol. 2009;9(11):757–66.

King C, Ilic A, et al. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. Cell. 2004;117(2):265–77.

King IL, Mohrs K, et al. A nonredundant role for IL-21 receptor signaling in plasma cell differentiation and protective type 2 immunity against gastrointestinal helminth infection. J Immunol. 2010;185(10): 6138–45.

Leonard WJ, Spolski R. Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. Nat Rev Immunol. 2005;5(9):688–98.

Linterman MA, Beaton L, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010;207(2):353–63.

Liu S, Lizee G, et al. IL-21 synergizes with IL-7 to augment expansion and anti-tumor function of cytotoxic T cells. Int Immunol. 2007;19(10):1213–21.

Liu L, Xu Y, et al. Upregulated IL-21 and IL-21 receptor expression is involved in experimental autoimmune uveitis (EAU). Mol Vis. 2009;15:2938–44.

Luzina IG, Atamas SP, et al. Spontaneous formation of germinal centers in autoimmune mice. J Leukoc Biol. 2001;70(4):578–84.

Maiti AK, Kim-Howard X, et al. Confirmation of an association between rs6822844 at the Il2-Il21 region and multiple autoimmune diseases: evidence of a general susceptibility locus. Arthritis Rheum. 2010;62(2):323–9.

McGuire HM, Vogelzang A, et al. A subset of interleukin-21+ chemokine receptor CCR9+ T helper cells target accessory organs of the digestive system in autoimmunity. Immunity. 2011a;34(4):602–15.

McGuire HM, Walters S, et al. Interleukin-21 is critically required in autoimmune and allogeneic responses to islet tissue in murine models. Diabetes. 2011b;60(3):867–75.

Monteleone G, Pallone F, et al. Interleukin-21 (IL-21)-mediated pathways in T cell-mediated disease. Cytokine Growth Factor Rev. 2009;20(2):185–91.

Nurieva R, Yang XO, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature. 2007;448(7152):480–3.

Nurieva RI, Chung Y, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity. 2008;29(1):138–49.

Ozaki K, Kikly K, et al. Cloning of a type I cytokine receptor most related to the IL-2 receptor beta chain. Proc Natl Acad Sci USA. 2000;97(21):11439–44.

Ozaki K, Spolski R, et al. A critical role for IL-21 in regulating immunoglobulin production. Science. 2002;298(5598):1630–4.

Ozaki K, Spolski R, et al. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. J Immunol. 2004;173(9):5361–71.

Ozaki K, Hishiya A, et al. Overexpression of interleukin 21 induces expansion of hematopoietic progenitor cells. Int J Hematol. 2006;84(3):224–30.

Parrish-Novak J, Dillon SR, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature. 2000; 408(6808):57–63.

Parrish-Novak J, Foster DC, et al. Interleukin-21 and the IL-21 receptor: novel effectors of NK and T cell responses. J Leukoc Biol. 2002;72(5):856–63.

Peluso I, Fantini MC, et al. IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes. J Immunol. 2007;178(2):732–9.

Pesce J, Kaviratne M, et al. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. J Clin Invest. 2006;116(7):2044–55.

Rankin AL, MacLeod H, et al. IL-21 receptor is critical for the development of memory B cell responses. J Immunol. 2011;186(2):667–74.

Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. Annu Rev Immunol. 2008;26:57–79.

Strengell M, Julkunen I, et al. IFN-alpha regulates IL-21 and IL-21R expression in human NK and T cells. J Leukoc Biol. 2004;76(2):416–22.

Sutherland AP, Van Belle T, et al. Interleukin-21 is required for the development of type 1 diabetes in NOD mice. Diabetes. 2009;58(5):1144–55.

Vinuesa CG, Cook MC, et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature. 2005;435(7041):452–8.

Vinuesa CG, Sanz I, et al. Dysregulation of germinal centres in autoimmune disease. Nat Rev Immunol. 2009;9(12):845–57.

Vogelzang A, King C. The modulatory capacity of interleukin-21 in the pathogenesis of autoimmune disease. Front Biosci. 2008;13:5304–15.

Vogelzang A, McGuire HM, et al. A fundamental role for interleukin-21 in the generation of T follicular helper cells. Immunity. 2008;29(1):127–37.

Wang XF, Yuan SL, et al. Changes of serum BAFF and IL-21 levels in patients with systemic lupus erythematosus and their clinical significance. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2007;23(11):1041–2.

Wei G, Wei L, et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity. 2009;30(1):155–67.

Wu Z, Kim HP, et al. Interleukin-21 receptor gene induction in human T cells is mediated by T-cell receptor-induced Sp1 activity. Mol Cell Biol. 2005;25(22):9741–52.

Yamamoto-Furusho JK, Miranda-Pérez E, Fonseca-Camarillo G, Sánchez-Muñoz F, Barreto-Zuñiga R, Dominguez-Lopez A. Interleukin 21 expression is increased in rectal biopsies from patients with ulcerative colitis. Inflamm Bowel Dis. 2010;16(7):1090.

Yang XP, Ghoreschi K, et al. Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions

of STAT3 and STAT5. Nat Immunol. 2011;12(3): 247–54.
Yi JS, Du M, et al. A vital role for interleukin-21 in the control of a chronic viral infection. Science. 2009;324(5934):1572–6.
Zeng R, Spolski R, et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. J Exp Med. 2005;201(1):139–48.
Zeng R, Spolski R, et al. The molecular basis of IL-21-mediated proliferation. Blood. 2007;109(10):4135–42.
Zhernakova A, van Diemen CC, et al. Detecting shared pathogenesis from the shared genetics of immune-related diseases. Nat Rev Genet. 2009;10(1):43–55.
Zotos D, Coquet JM, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med. 2010;207(2): 365–78.

Immunodeficiency in Autoimmune Diseases

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Abbreviations

AHA Autoimmune hemolytic anemia
BAFF-R B cell activating factor receptor
Btk Bruton's tyrosine kinase
CGD Chronic granulomatous disease
CMV Cytomegalovirus
CVID Common variable immunodeficiency
EBV Epstein-Barr virus
FHL Familial hemophagocytic lymphohistiocytosis
GIT Gastrointestinal tract
GUT Genito urinary tract
HLH Hemophagocytic lymphohistiocytosis
IBD Inflammatory bowel disease
ICOS Inducible T cell costimulatory
Ig Immunoglobulin
IgAD IgA deficiency
ITP Immune thrombocytopenia
LIP Lymphoid interstitial pneumonia
MGD Midline granulomatous disease
MSH-5 MutS protein homolog 5
NADPH Nicotinamide adenine dinucleotide phosphate
RAG Recombination activating genes
SLAM Signaling lymphocyte-activation molecule
TACI Transmembrane activator and calcium-modulator and cyclophilin ligand interactor
TCR T cell receptor
VZV Varicella-zoster virus
WAS Wiskott-Aldrich syndrome
XLA X-linked agammaglobulinemia
XLP X-linked lymphoproliferative syndrome

Synonyms

Autoimmune disorder; Immune deficiency

Definition

Immunodeficiency is a state in which the immune system's ability to fight infections is compromised due to abnormal function of immune system components.

In autoimmunity the immune system turns against the host's own tissues, cells, or cell components, resulting in prolonged inflammation and subsequent tissue destruction.

Genetic Predisposition to Autoimmunity

Interest in the role immunodeficiency may play in the development of autoimmunity in humans has been revived in recent years. Large-scale genetic association studies, like GWAS (genome-wide association studies) and whole genome sequencing, have highlighted the importance of genetics in the development of autoimmunity. This is confirmed by familial clustering and higher rates of concordance in monozygotic compared to dizygotic twins in patients with autoimmune disorders. Although contribution of the genetic component in each disorder is not equal (e.g., monozygotic twin

concordance is 5 % in systemic sclerosis and 40 % in type 1 diabetes mellitus (T1DM)) it represents a key element in all of them.

A large number of genes have been found to contribute to the development of autoimmune disorders in recent years (Tables 1 and 2). The vast majority of those are genes related to the immune system. Although the effect gene polymorphisms have on the immune system's development and function has not been delineated yet, it is hypothesized that, at least in some cases, these variations may allow for a minor immunodeficiency state to develop (Grammatikos and Tsokos 2012). In contrast to primary immunodeficiency syndromes which are associated with severe single-gene defects and result in outright immune system dysfunction, the widespread single nucleotide polymorphisms and gene variants seen in autoimmune patients seem to result in subtler immune system abnormalities. The cumulative effect of these gene polymorphisms seems to be an immune system with decreased capacity to fight infections.

Infections in Autoimmune Diseases

Patients in most autoimmune disorders experience a higher rate of infections than healthy individuals (Sfriso et al. 2010). Even opportunistic pathogens normally observed in immunocompromised patients (e.g., *Toxoplasma gondii, Pneumocystis carinii*, and *Cryptococcus neoformans*) are encountered in some cases. Although traditional risk factors, like immunosuppressant therapy, certainly contribute, they do not seem to be sufficient to explain this increased susceptibility. The higher frequency of immune system defects in these patients provides a more likely explanation.

Indeed, multiple links between infectious microorganisms and almost all autoimmune disorders have been discovered (Tables 1 and 2). Although what drives this association is still uncertain, it is postulated that a lowered immune system defense level may be allowing for multiple infections to develop and for inflammation to persist. These infections, both at a clinical and a subclinical level, may represent the primary trigger for the development of autoimmunity (Fig. 1). Established examples of autoimmunity that follows infection include rheumatic fever presenting after *Streptococcus pyogenes* infection and Guillain-Barre syndrome after *Campylobacter jejuni*, *HIV*, *CMV*, or *Hemophilus influenza infection.*

A number of pathways exist that can allow a localized inflammation to spread systemically and an immune response against a foreign antigen to turn toward self-antigens (Munz et al. 2009). Among those, epitope spreading, molecular mimicry, bystander, and superantigen activation. Epitope spreading refers to the ability of antigen presenting cells to present multiple antigenic determinants from a single foreign agent's protein and not just the one responsible for their activation, allowing for multiple T cell-specificities to be activated. Superantigens, on the other hand, are antigens that have the inherent property to directly cross-link multiple T cell receptors and activate multiple T cell clones. In bystander activation, it is apoptotic material from host cells presented by antigen presenting cells in the context of inflammation that are believed to allow for autoreactive T cells to develop. Finally, molecular mimicry refers to the process of generation of autoreactive T cell clones owing to sequence similarities of self-antigens with foreign epitopes.

Despite the fact infectious diseases are very common, autoimmunity only develops in a small fraction of the population. Existence of an underlying immune dysregulation in the subgroup of individuals that develop autoimmunity could explain this disparity. Autoimmunity is also commoner than generally thought: although only 3 % of the population is affected by autoimmune diseases, many more are found to harbor autoantibodies without any clinical manifestations.

Apart from genetic predisposition, it seems that environmental influences (e.g., diet or psychology) alter the manifestation of autoimmunity in each patient. Exposure to these factors is thought to begin long before disease development. For instance, a number of reports have been published on the presence of autoantibodies

Immunodeficiency in Autoimmune Diseases, Table 1 Genetic polymorphisms and microorganisms linked to autoimmune disorders

Systemic diseases	Associated genes	Associated infections
▶ Ankylosing spondylitis	HLAB27, NOD2, TNFa, MMP3, TGFβ, IL1	*Enterobacteria, Chlamydia, Shigella, Salmonella, Klebsiella pneumoniae*
Autoimmune cytopenias (anemia, thrombocytopenia, neutropenia)	HLAA2, HLADRB1, FCGR2A, FCGR3A	*Helicobacter pylori, HIV, HCV*
Myasthenia gravis	PTPN22, CTLA4, HLAB8, HLADR3, HLADR1, FCGR2A, FCGR3A, FCGR3B, IL10	*HCV, HSV*
Polyglandular autoimmune syndrome type I	AIRE	*Candida albicans, Rubella*
Rheumatoid arthritis	STAT4, IRF5, UBE2L3, HLADR4, TRAF1, REL, PTPN22, CTLA4, BLK, CCL21, CD40, IL2, PRKCQ, CD28, PDCD1, TNFSF14	*EBV, CMV, HCV, Escherichia Coli, Proteus* sp., *Mycobacteria, Parvovirus B19*
Scleroderma	HLADRB1, HLADQB1, STAT4, TBX21, IRF5, PTPN22, BANK1, BLK, CD247	*EBV, CMV, Parvovirus B19, H. pylori*
Sjögren's syndrome	HLADR3, IRF5, IL10, IL1, MECP2	*EBV, H. Pylori, Coxsackie B3*
Systemic lupus erythematosus	C2,C4A, C4B and C1q deficiencies, FCGR3B, STAT4, TREX1, TLR 7/8, IRAK1, SLC15A4, IFIH1, IRF5, STAT4, STAT1, TYK2, UBE2L3, HLADR2, PD1, PTPN22, PDCD1	*EBV, CMV, Rubella, Parvovirus B19*
Giant cell arteritis/ polymyalgia rheumatica	HLADRB1, RANTES, ICAM1, IL1 RN	*EBV, Chlamydia pneumoniae, Parvovirus B1, HHV*
Granulomatosis with polyangiitis and microscopic polyarteritis	IL10, IL7R, NOTCH1, ITGA2	*EBV, CMV, Toxoplasma, Parvovirus B19, Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenzae*
Organ-specific diseases	Associated genes	Associated infections
Addison's disease (idiopathic)	CIITA, CTLA4, HLADR3, PTPN22, HLADR, HLADQ	
Autoimmune hepatitis	HLAB8, HLAB14, HLADR3, HLADR4, HLADW3, C4 deficiency, CD45	*Measles, HCV, HBV, HSV, VZV, CMV, and EBV*
Autoimmune thyroiditis (Graves' and Hashimoto's disease)	CTLA4, IFIH1, PTPN22, HLADR5, HLADR3	*HCV, HIV, H. pylori, Campylobacter jejuni, Yersinia enterocolitica*
Celiac disease	HLADQ2, HLADQ8, IRF4, UBE2L3, CTLA4, TNFSF14	*HCV, Adenovirus 12, Rotavirus, Human intestinal adenovirus*
IBD (Crohn's disease and Ulcerative colitis)	NOD2, CARD9, IRF5, STAT4, STAT3, JAK2, UBE2L3, TNFSF15, FCGR2A, ORMDL3	*HCV, CMV, H. Pylori, Y. enterocolitica, Mycobacterium avium paratuberculosis, C. difficile*
Multiple sclerosis	CLEC16A, IRF5, IRF8, STAT1, STAT3, TYK2, HLADR2	*EBV, HSV6, Measles, Chlamydophila pneumoniae*
Psoriasis	DEFB4 (high copy number), STAT4, HLAB13, HLAB17, HLACW6	*Group A β-hemolytic streptococcus, HIV, CMV*
Type 1 diabetes	IFIH1, CLEC16A, STAT4, TYK2, CTLA4, PTPN22, HLADQ8, HLADQ2, HLADQ6, CD247	*Enterovirus, Rotavirus, CMV, Mumps, Rubella*

Immunodeficiency in Autoimmune Diseases, Table 2 Genetic polymorphisms linked to autoimmune disorders: role of the encoded proteins

Gene	Full name	Function of encoded protein
AIRE	Autoimmune regulator	Transcription factor responsible for expression of self-antigens in thymus
BANK1	B cell scaffold protein with ankyrin repeats	Involved in BCR-induced calcium mobilization
BLK	B lymphoid tyrosine kinase	Involved in cell proliferation and differentiation
C1q, C2, C4	Complement components of the classical pathway	Involved in protection from bacteria, causing chemotaxis, opsonization, cell lysis, etc.
CARD9	Caspase recruitment domain-containing protein	Involved in TLR-NOD2 signaling
CCL21	Chemokine (C-C motif) ligand 21	Chemokine involved in attracting thymocytes and activated T cells
CD247	CD3ζ chain	Cell signaling molecule involved in TCR signaling
CD28	T-cell-specific surface glycoprotein	Activating surface costimulatory molecule found on T cells
CD40	TNF receptor superfamily member 5	Costimulatory molecule on antigen presenting cells
CD45	Protein tyrosine phosphatase	Different CD45 isoforms expressed by naïve, effector cells, and memory T cells
CIITA	Class II transactivator	Transcriptional coactivator of MHCII genes
CLEC16A	C-type lectin domain family 16 member A	C-type lectin that contains carbohydrate recognition domains
CTLA4	Cytotoxic T lymphocyte antigen 4	Inhibitory cell surface costimulatory molecule found on T cells
DEFB4	Beta-defensin 4	Secreted antimicrobial peptide
FCGR 2A/3A/3B	Immunoglobulin G Fc receptor	Cell surface receptor for antibodies
HLA	Human leukocyte antigen	Cell surface molecule that presents antigens to T cells
ICAM1	Intercellular adhesion molecule 1	Cell surface glycoprotein functioning in lymphocyte tissue transmigration
IFIH1	Interferon-induced helicase	Enzyme that contributes to apoptosis of virally infected cells
IL1	Interleukin 1	Cytokine with role in inflammation, bone remodeling, insulin secretion, etc.
IL1 RN	IL1 receptor antagonist	Inhibits the activities of interleukin 1, alpha (IL1A), and interleukin 1, beta (IL1B)
IL2	Interleukin 2	Necessary for the growth of T cells and the maintenance of T regulatory cells
IL7R	Interleukin 7 receptor	Involved in V(D)J recombination during lymphocyte development
IL10	Interleukin 10	Antiinflammatory cytokine/stimulates B cell maturation and antibody production
IRAK1	Interleukin 1 receptor-associated kinase	Protein kinase that links TLR signaling to TRAF6
IRFs	Interferon regulatory factors	Transcription factors involved in virus-mediated activation of interferon production pathways
ITGA2	Integrin alpha 2	Cell surface protein involved in cell adhesion
JAK2	Janus kinase 2	Involved in transmitting signals from various cytokines, growth hormones, etc.
MECP2	Methyl-CpG-binding protein 2	Binds to methylated DNA/transcriptional repressor and activator
MMP3	Matrix metalloproteinase-3	Involved in the breakdown of extracellular matrix
NOD2	Nucleotide binding and oligomerization domain	Cytoplasmic receptor for peptidoglycans on bacterial cell walls

(*continued*)

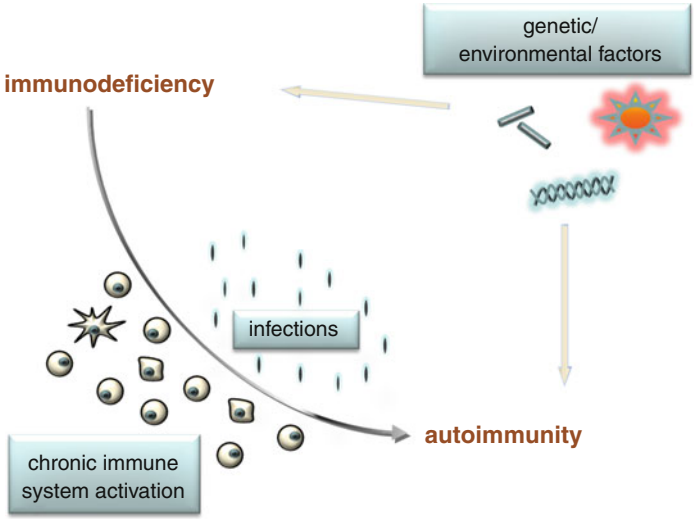
Immunodeficiency in Autoimmune Diseases, Table 2 (continued)

Gene	Full name	Function of encoded protein
NOTCH1	Notch homolog 1, translocation-associated	Plays multiple roles in tissue development/induces T cell proliferation
ORMDL3	ORM1-like protein 3	Believed to regulate endoplasmic reticulum-mediated Ca^{+2} signaling
PD1	Programmed death 1	Membrane protein that negatively regulates immune responses
PDCD1	Programmed cell death protein 1	Cell surface protein/plays a role in B cell differentiation
PRKCQ	Protein kinase C theta	Phosphorylates various proteins/important for T cell activation
PTPN22	Protein tyrosine phosphatase, nonreceptor type 22	Protein tyrosine phosphatase that alters the responsiveness of T and B cell receptors
RANTES	Regulated upon activation, normal T cell expressed, and presumably secreted	Chemokine that induces chemotaxis of T cells, ▶ eosinophils, and basophils
REL	v-rel reticuloendotheliosis viral oncogene homolog	Member of the NF-κB family of transcription factors
SLC15A4	Solute carrier family 15, member 4	Transporter of NOD1 ligands in early endosomes
STATs	Signal transducers and activators of transcription	Transcription factors activated in response to cytokines and growth factors
TBX21	T cell specific T-box transcription factor	Transcription factor that regulates Th1/Th2 balance
TGFβ	Transforming growth factor beta	Cytokine involved in regulation of cell cycle, differentiation, and apoptosis
TLRs	Toll-like receptors	Recognize specific components conserved among microorganisms
TNFa	Tumor necrosis factor a	Pleiotropic inflammatory cytokine
TNFSFs	Tumor necrosis factor superfamily ligands	Stimulate the proliferation of T cells/ promote activation of caspases and apoptosis
TRAF1	TNF receptor-associated factor 1	Mediates signal transduction from receptors of the TNFR superfamily
TREX1	Three prime repair exonuclease 1	3–5 exonuclease responsible for proofreading during genome replication
TYK2	Tyrosine kinase 2	Tyrosine kinase implicated in cytokine signaling
UBE2L3	Ubiquitin-conjugating enzyme E2L 3	Degradation of NFκB precursor/suppression of innate immune response(?)

several years prior to disease development in systemic lupus erythematosus (SLE) (Reeves et al. 2011). Increased susceptibility to infection in these patients may very well exist during this long period of preexisting autoimmunity. It is possible that exposure to infections and other environmental factors occurring in a discontinuous fashion may fuel disease flares.

Common Immune Defects in Autoimmune Diseases

A number of evidence shows that the current organization of autoimmune disorders in isolated disease entities may have little to do with underlying pathogenesis. Patients belonging to different autoimmune disorders frequently exhibit common characteristics and overlapping symptoms. Also patients suffering from organ-specific diseases sometimes present with systemic features and vice versa. On the other hand, considerable phenotypic heterogeneity is sometimes encountered within the same disorder, for example, SLE patients can present with as mild a picture as rash and arthritis to as severe as end-stage renal disease and psychosis (Tsokos 2011). This evidence shows that entirely different autoimmune diseases may actually have common etiopathogenic origins. Under this notion, autoimmunity may



Immunodeficiency in Autoimmune Diseases, Fig. 1 From autoimmunity to immunodeficiency. Schematic representation of the proposed link between immunodeficiency and autoimmunity. Subtle immune system abnormalities that accumulate in patients predisposed to autoimmunity may result in a decreased host defense level and allow for more frequent infections to develop. Infectious microorganisms along with the chronic immune system activation ensuing may represent the primary trigger for the development of autoimmunity. Both environmental triggers and other genetic factors seem to contribute to shaping autoimmunity in each individual patient

represent the common denominator with varying clinical phenotypes stemming from individual environmental and genetic contributions.

Indeed, a number of genetic polymorphisms and defects have been found that are associated to not only one, but multiple autoimmune disorders (Table 3). Although for most cases the exact role of these genetic defects in the development of autoimmunity is not certain, some hypotheses have been made based on the function of the encoded proteins. Stemming from this knowledge, defects can be categorized in those associated with suppressed immunity, those associated with an increased immune activation state, and those associated with escape from tolerance.

Polymorphisms Associated with Suppressed Immunity

PTPN22 (protein tyrosine phosphatase nonreceptor 22) polymorphisms are associated with T1DM, rheumatoid arthritis (RA), SLE, scleroderma, myasthenia gravis, Addison's disease, and autoimmune thyroiditis (Rioux and Abbas 2005; Gregersen and Behrens 2006). Together with CSK (an intracellular tyrosine kinase), PTPN22 is responsible for downregulating the action of LCK (another tyrosine kinase). Since LCK is involved in T cell receptor (TCR) signaling, PTPN22 can essentially turn off TCR signaling. PTPN22 polymorphisms associated with autoimmunity have been found to result in an increased threshold of stimulation for TCR signaling. Although the effect of this defect on the immune system is uncertain, decreased TCR signaling may result in infectious agents' antigens to escape recognition.

Another molecule associated to multiple autoimmune diseases is CD3ζ chain (or else CD247). CD3ζ chain constitutes part of the TCR signaling machinery and is responsible for transmitting signals downstream upon antigen recognition. Low expression of CD3ζ chain is found in SLE, T1DM, and scleroderma (Tsokos 2011). Defective TCR signaling has been shown to lead to the development of a lethal, multiorgan autoimmune disease in mice. Mutations in the gene encoding for ZAP70, a downstream signaling target of the CD3ζ complex, also lead to the development of

Immunodeficiency in Autoimmune Diseases, Table 3 Common genetic polymorphisms in autoimmune disorders.

Gene	Associated autoimmune disorders	Function of encoded protein	Result of polymorphism
Associated with suppressed immunity			
PTPN22	Type 1 diabetes, rheumatoid arthritis, SLE, myasthenia gravis, and autoimmune thyroiditis	Turns off TCR signaling	Increased threshold of stimulation for TCR signaling
CD247	SLE, type 1 diabetes, and scleroderma	Transmits signals upon Ag recognition	Defective signaling upon Ag recognition
UBE2L3	Rheumatoid arthritis, SLE, celiac disease, and inflammatory bowel disease	Marks abnormal proteins for degradation	Increased levels of NF-κB/inflammation(?)
RUNX1	Psoriasis, rheumatoid arthritis, and SLE	Transcription factor	Altered expression levels of PDCD1, SLC22A4 SLC9A3R1, and NAT9 genes
FCGR2A, FCGR3A, FCGR3B	Inflammatory bowel disease, autoimmune cytopenias, myasthenia gravis and SLE	Bind the Fc portion of IgG antibodies	Increased susceptibility to infections(?)
Associated with increased immune activation state			
SIAE	Multiple	Regulates inhibitory signals	Enhanced B cell receptor activation
FOXP3	Type I diabetes, thyroiditis, inflammatory bowel disease, atopic dermatitis (IPEX)	T regulatory cell master regulator	Expansion of autoreactive T cells in the periphery
CTLA4	Type 1 diabetes, rheumatoid arthritis, autoimmune thyroiditis, Addison's disease and celiac disease	Inhibitory signaling costimulatory molecule	Enhanced T cell activation
IRF5	Rheumatoid arthritis, scleroderma, Sjögren's syndrome, SLE, and IBD	Involved in the production of type I interferons	Higher serum IFNα activity
Associated with escape from tolerance			
AIRE	Autoimmune hypothyroidism, Addison's disease (APS1)	Regulates thymic expression of self-antigens	Escape from negative selection of self-reactive T cell clones
TNFRFS6, TNFFS6, CASP10, CASP8	Autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, autoimmune neutropenia (ALPS)	Involved in apoptosis	Failure to delete autoreactive T cells
HLA	Multiple	Antigen presentation	Alteration in peptide affinity

autoimmunity in mice. Infectious agents seem to play an important role because mice that are bred in a microbial-free environment are salvaged from autoimmunity. Low CD3ζ chain expression may result in defective signaling upon foreign antigen recognition and decreased immune response to infectious agents.

UBE2L3 (ubiquitin-conjugating enzyme E2 L3) polymorphisms have been associated with RA, SLE, celiac disease, and inflammatory bowel disease (Wang et al. 2012). UBE2L3 is an enzyme involved in ubiquitination, that is, the process of marking abnormal or short-lived proteins for degradation by conjugating them with ubiquitin. This enzyme has been shown to participate in the ubiquitination of many molecules that are important in immune cells, for example, c-Fos and the NF-κB precursor p105. Cells that carried the risk haplotype were found to have higher levels of UBE2L3 mRNA and its encoded protein. Since NF-κB is upregulated in response to inflammation and UBE2L3 decreases the levels of NF-κB, this variant may result in a diminished innate immune response and a higher rate of infections.

Another example is transcription factor RUNX1 (runt-related transcription factor 1). In three independent studies RUNX1 binding site variants have been associated to three different autoimmune diseases (Wong et al. 2012). The genes that have been found to be associated are PDCD1 with SLE, SLC22A4 with RA and both SLC9A3R1 and NAT9 with psoriasis. Although the precise mechanisms that underlie these associations are not yet clear, disrupted binding of RUNX1 to these genes seems to affect their expression levels. PDCD1 encodes for a cell surface protein that seems to play an important role in B cell development, SLC22A4 for a sodium-ion dependent carnitine transporter, SLC9A3R1 for a sodium/hydrogen exchanger regulatory cofactor, and NAT9 for an acetyltransferase.

Various genes encoding for Fc gamma receptors (FCGRs) have been associated to autoimmune disorders: FCGR2A with inflammatory bowel diseases, autoimmune cytopenias and myasthenia gravis, FCGR3A with autoimmune cytopenias and myasthenia gravis, and FCGR3B with SLE and myasthenia gravis (Fossati et al. 2001). Fc gamma receptors are cell surface receptors that bind the Fc portion of IgG antibodies, thus recognizing IgG opsonized particles. Upon binding, cells respond by releasing inflammatory mediators, initiating phagocytosis and activating antibody-dependent cell-mediated cytotoxicity. These processes serve to eliminate invading pathogens. Although the exact role of the above polymorphisms in disease is yet to be determined, an interesting similarity is that RA, SLE, autoimmune cytopenias, and myasthenia gravis are all antibody-mediated diseases. Furthermore, certain Fc gamma receptor polymorphisms have been associated in the past with increased susceptibility to infections.

Polymorphisms Associated with an Increased Immune Activation State

An interesting molecule that has been associated with many autoimmune disorders is SIAE (sialic acid acetylesterase). SIAE is an enzyme that removes acetyl moieties and thus regulates sialic acid molecules. Defective or loss-of-function mutations of SIAE are identified more commonly in individuals suffering from autoimmune disorders than in controls. Mutation of SIAE in mice causes enhanced B cell receptor activation in peripheral B cells and the development of antichromatin autoantibodies and glomerular immune complex deposits (Cariappa et al. 2009). CD22, a sialic acid-recognizing molecule that transmits inhibitory signals and is constitutively expressed on mature B cells, seems to play a role in this. In SIAE mutations inhibitory signaling from CD22 is suppressed and B cells become hyper-activated.

CTLA4 (cytotoxic T lymphocyte antigen 4) polymorphisms have been associated with T1DM, RA, autoimmune thyroiditis, myasthenia gravis, Addison's disease, and celiac disease (Rioux and Abbas 2005; Gregersen and Behrens 2006). The human risk haplotype is associated with lower levels of a splice variant that encodes for soluble CTLA4. Since CTLA4 is a costimulatory molecule involved in transmitting inhibitory signals to T cells, it seems that these variants may allow for a higher T cell activation state to develop.

IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) is a syndrome where T1DM, thyroiditis inflammatory bowel disease, atopic dermatitis, and infections coexist. Mutations in FOXP3 (forkhead box P3), the master regulator of T regulatory cells, are known to be responsible for the development of IPEX (Rioux and Abbas 2005). It is well established that regulatory T cells are crucial for decreasing the activation status of various immune system components.

Scleroderma, RA, Sjögren's syndrome, SLE, and inflammatory bowel disease are all examples of diseases in which IRF5 (interferon regulatory factor 5) gene polymorphisms are seen (Gregersen and Behrens 2006). IRF5, or interferon regulatory factor 5, is a transcription factor involved in the production of type I interferons. The engagement of either toll-like receptor (TLR) 7 or TLR8 by single-stranded RNA induces IRF5 activation and subsequent transcription of multiple proteins involved in antiviral defense. Presence of the IRF5 risk variant seems to be associated with a higher serum IFNα activity. High IFNα levels are in turn accused of being central to the pathogenesis of multiple autoimmune diseases, including RA and SLE.

Polymorphisms Associated with Escape from Tolerance

Most autoimmune diseases are strongly associated to one or more HLA (human leukocyte antigen) polymorphisms (Walker and Nepom 2005; Delves et al. 2011). HLA molecules enable antigen presentation and subsequent T cell activation through the HLA–TCR interaction. It has been demonstrated that even one amino acid change in the structure of HLA can cause dramatic alterations in its antigen binding affinity and efficiency of T cell induction. Although this has not been proven yet, such variations may allow self-antigens to induce strong T cell activation and autoimmunity to develop.

Two autoimmune syndromes that have been found to be associated with escape from tolerance are autoimmune lymphoproliferative syndrome (ALPS) and autoimmune polyendocrine syndrome 1 (APS1). ALPS is a syndrome where autoimmune manifestations (hemolytic anemia, thrombocytopenic purpura, and neutropenia) are combined with lymphadenopathy, splenomegaly, and hypergammaglobulinemia (Rieux-Laucat et al. 2003; Vaux 2006). There are multiple forms of ALPS but all of them are associated to some kind of defect in apoptotic pathways. Apoptotic pathways are known to be crucial in the process of central tolerance and autoreactive T/B cell clone deletion. Absence of this process is hypothesized to be responsible for the development of autoimmunity in these patients.

Mutations in AIRE, a gene that encodes for a transcription factor that regulates thymic expression of self-antigens, have been found to be responsible for APS1 (Rioux and Abbas 2005; Lankisch et al. 2009). Expression of self-antigens in the thymus serves as an effective mechanism to avoid the escape of autoreactive T cells in the periphery. In AIRE mutations thymic deletion of autoreactive cells is impaired and multiorgan autoimmunity occurs. Autoimmune hypothyroidism, Addison's disease, and chronic *Candida* infection are the commonest manifestations encountered in these patients. Clinical phenotype is quite diverse, though, implicating an important role for other environmental factors, like infections.

Autoimmune Manifestations in Immunodeficiency Disorders

Not only autoimmunity is linked to immunodeficiency, but the reverse also seems to hold true. Autoimmune phenomena are part of many classic immunodeficiency disorders and a link with specific infections and the development of chronic inflammation has been postulated (Table 4) (Grammatikos and Tsokos 2012).

X-linked lymphoproliferative (XLP) syndrome is one example of an immunodeficiency disorder associated with multiple autoimmune phenomena. XLP is caused by mutations in the gene encoding for SLAM associated protein (SAP), involved in CD8+ T and NK cell signaling pathways (Chapel et al. 2006). The result of this mutation is uncontrolled EBV infection and widespread immune system activation with a hemophagocytic lymphohistiocytosis picture. Fever, hepatosplenomegaly, lymphadenopathy, jaundice, and rash along with laboratory findings of lymphocytosis and histiocytosis are seen in these patients.

A similar example is familial hemophagocytic lymphohistiocytosis (FHL). FHL is a syndrome that is characterized by uncontrolled immune system activation and chronic inflammation. It presents as prolonged fever, rashes, pancytopenia, lymphadenopathy, and hepatosplenomegaly. Half of cases of FHL are known to be caused by mutations in PRF1 and UNC13D genes, two genes that encode for proteins involved in cell cytolytic functions. Cytolysis is an important function in the process of destroying viral-infected cells and these patients have frequent viral infections.

Wiskott–Aldrich syndrome (WAS) is another immunodeficiency syndrome where multiple autoimmune manifestations are encountered. WAS protein (WASp), a protein involved in actin polymerization, is found to be defective in these patients (Cruse 2004). Defective actin polymerization is thought to result in abnormal immunological synapse formation in T cells upon TCR signaling. Apart from recurrent bacterial infections, these patients also exhibit eczema, ITP (immune thrombocytopenia), and AHA (autoimmune hemolytic anemia).

Immunodeficiency in Autoimmune Diseases, Table 4 Autoimmune manifestations in primary immunodeficiency syndromes (Adapted from Grammatikos AP, Tsokos GC (2012))

Disease	Defective molecule (involved in)	Infectious phenomena	Autoimmune/lymphoproliferative manifestations
FHL	*PRF1, UNC13D* (T/NK cell cytotoxicity)	Increased viral infections	HLH (inflammation and tissue destruction)
XLP	*SAP* (CD8+ T/NK cell signaling pathways)	Uncontrolled EBV infection	HLH (inflammation and tissue destruction), lymphoma
WAS	*WASP* (actin cytoskeleton)	Recurrent bacterial infections	ITP, AHA, vasculitis, eczema, lymphoma
CGD	NADPH oxidase (Neutrophil bactericidal mechanisms)	Recurrent bacterial/fungal infections	Chronic gut/lung/GUT inflammation
MGD	*RAG1*, *RAG2* (somatic rearrangement of Ig and TCR genes)	Systemic viral infections (CMV, EBV, VZV)	Caseating granulomas of the nose, sinuses, palate, and upper airways
CVID	*TACI, ICOS, BAFF-R*, *CD19*, *MSH-5* (antibody production)	Recurrent sinopulmonary, GIT infections	IBD, ITP, AHA, LIP, lymphoma
IgAD	*IgA1*, *IgA2*, and *MHC* genes (IgA antibody production)	Recurrent infections	SLE, rheumatoid arthritis, ITP, allergy, asthma
XLA	*Btk* (B cell development/signaling)	Recurrent infections	Arthritis, AHA, scleroderma, type 1 diabetes

Patients with chronic granulomatous disease have defective NADPH (nicotinamide dinucleotide phosphate) oxidase, the enzyme complex responsible for the generation of superoxide and other reactive oxygen species in phagocytic cells (Kumararatne 2009). Inability of phagocytes to produce bactericidal oxidants results in the development of recurrent bacterial and fungal infections. A hallmark of the disease is the development of granulomatous inflammation of hollow viscera; examples are inflammatory bowel disease, urethral strictures, and bladder granulomas.

A recent study reported the presence of hypomorphic RAG (recombination activating gene) mutations in patients suffering from midline granulomatous disease (De Ravin et al. 2010). RAG encodes for enzymes that play an important role in the rearrangement and recombination of the immunoglobulin and TCR genes. In contrast to complete loss of gene function seen in severe combined immunodeficiency syndrome (SCID), hypomorphic RAG mutations allow for 50 % activity of the encoded protein. As a consequence, these patients experience less severe infections and survive longer. In contrast to SCID, their phenotype is primarily autoimmunity-based (e.g., caseating granulomas of the nose, sinuses, palate, and upper airways).

Common variable immune deficiency (CVID) is another typical immunodeficiency syndrome with multiple autoimmune symptoms (Chapel et al. 2006). A number of different genes have been associated to the development of CVID: TACI (transmembrane activator and CAML interactor), ICOS (inducible costimulatory), BAFF-R (B cell activating factor receptor), MSH-5 (mutS homolog 5), and CD19. What these genes have in common is that they all are involved in antibody production. CVID patients exhibit characteristic hypogammaglobulinemia and experience frequent infections. However, autoimmune phenomena are also very commonly seen; for example, inflammatory bowel disease, immune thrombocytopenia, autoimmune haemolytic anemia, and lymphocytic interstitial pneumonitis.

Although patients with IgA deficiency are usually asymptomatic they sometimes exhibit increased frequency of infections, particularly of the respiratory, digestive, and genitourinary systems (Kumararatne 2009). Autoimmune and atopic phenomena like SLE, RA, ITP, allergy, and asthma are also occasionally seen. A higher prevalence of autoimmune manifestations is moreover seen in unaffected relatives of these patients. Interestingly, patients diagnosed with

autoimmune diseases such as SLE and RA are more frequently found to have IgA deficiency than the general population.

A defect in Btk (Bruton's tyrosine kinase), a gene involved in B cell maturation, is responsible for XLA (X-linked agammaglobulinemia) (Chapel et al. 2006). Patients with XLA present with low or absent B cells and low immunoglobulin levels. As a result, recurrent pneumonia, otitis media, diarrhea, and other infectious diseases are experienced by these individuals. Autoimmunity is also frequently seen with arthritis, AHA, scleroderma, and T1DM being the most common manifestations.

Conclusions

Although immunodeficiency and autoimmunity have long been considered as two separate disease entities, it is now becoming evident that they have many characteristics in common and may, at least in some cases, be interconnected. It has long been known that patients with primary immunodeficiency syndromes experience multiple autoimmune phenomena. More recently, it is starting to be recognized that immunodeficiency may represent an important contributor to the development of autoimmune disorders. The common denominator for these findings seems to be an increased preponderance to infections and a subsequent constant state of inflammation. The severity of the genetic abnormality may determine whether a predisposed individual exhibits an immunodeficiency- or an autoimmunity-based phenotype. Future research is expected to help better understand the links between those two disease entities and shed more light into their shared characteristics.

Cross-References

- ▶ Epigenetics in Autoimmunity
- ▶ Giant Cell Arteritis
- ▶ Psoriasis
- ▶ PTPN22
- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Cariappa A, Takematsu H, Liu H, Diaz S, Haider K, Boboila C, et al. B cell antigen receptor signal strength and peripheral B cell development are regulated by a 9-O-acetyl sialic acid esterase. J Exp Med. 2009;206:125–38.

Chapel H, Haeney M, Misbah S. Immunodeficiency. Oxford: Blackwell; 2006.

Cruse JM. Immunodeficiencies: congenital and acquired. In: Cruse JM, Lewis RE, Wang H, editors. Immunology guidebook. San Diego: Elsevier; 2004. p. 465–74.

De Ravin SS, Cowen EW, Zarember KA, Whiting-Theobald NL, Kuhns DB, Sandler NG, et al. Hypomorphic Rag mutations can cause destructive midline granulomatous disease. Blood. 2010;116:1263–71.

Delves PJ, Martin SJ, Burton DR. Roitt IM Roitt's essential immunology. Oxford: Blackwell; 2011.

Fossati G, Bucknall RC, Edwards SW. Fcgamma receptors in autoimmune diseases. Eur J Clin Invest. 2001;31:821–31.

Grammatikos AP, Tsokos GC. Immunodeficiency and autoimmunity: lessons from systemic lupus erythematosus. Trends Mol Med. 2012;18:101–8.

Gregersen PK, Behrens TW. Genetics of autoimmune diseases – disorders of immune homeostasis. Nat Rev Genet. 2006;7:917–28.

Kumararatne DS. Immunological aspects of immunodeficiency diseases. In: Zabriskie JB, editor. Essential clinical immunology. New York: Cambridge University Press; 2009. p. 61–91.

Lankisch TO, Jaeckel E, Strassburg CP. The autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy or autoimmune polyglandular syndrome type 1. Semin Liver Dis. 2009;29:307–14.

Munz C, Lunemann JD, Getts MT, Miller SD. Antiviral immune responses: triggers of or triggered by autoimmunity? Nat Rev Immunol. 2009;9:246–58.

Reeves WH, Xu Y, Zhuang H, Li Y, Yang L. Origins of antinuclear antibodies. In: Lahita RG, Tsokos GC, Buyon JP, Koike T, editors. Systemic lupus erythematosus. Oxford: Academic; 2011. p. 213–35.

Rieux-Laucat F, Le Deist F, Fischer A. Autoimmune lymphoproliferative syndromes (ALPS). In: Herrmann M, Kalden JR, editors. Apoptosis and autoimmunity. Darmstadt: Wiley-VCH; 2003. p. 135–57.

Rioux JD, Abbas AK. Paths to understanding the genetic basis of autoimmune disease. Nature. 2005;435:584–9.

Sfriso P, Ghirardello A, Botsios C, Tonon M, Zen M, Bassi N, et al. Infections and autoimmunity: the multifaceted relationship. J Leukoc Biol. 2010;87:385–95.
Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365:2110–21.
Vaux DL. Apoptosis and autoimmunity: lymphoproliferative syndromes. In: Rose NR, Mackay IR, editors. The autoimmune diseases. St. Louis: Elsevier/Academic; 2006.
Walker MR, Nepom G. Major histocompatibility complex and autoimmunity. In: Rose NR, Mackay IR, editors. The autoimmune diseases. San Diego: Elsevier; 2005. p. 47–58.
Wang S, Adrianto I, Wiley GB, Lessard CJ, Kelly JA, Adler AJ, et al. A functional haplotype of UBE2L3 confers risk for systemic lupus erythematosus. Genes Immun. 2012;13(5):380–7.
Wong WF, Kohu K, Nakamura A, Ebina M, Kikuchi T, Tazawa R, et al. Runx1 deficiency in CD4+ T cells causes fatal autoimmune inflammatory lung disease due to spontaneous hyperactivation of cells. J Immunol. 2012;188:5408–20.

Immunoglobulin Somatic Hypermutation and Class-Switch DNA Recombination

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Synonyms

Somatic hypermutation (SHM): Immunoglobulin (antibody) somatic hypermutation; Somatic hypermutation of antibody genes; Somatic hypermutation of immunoglobulin genes
Class-switch DNA recombination (CSR): Class-switch recombination; Immunoglobulin (antibody) class switching

Definition

Somatic hypermutation. During B cell responses to antigen, immunoglobulin (Ig) variable region DNA that encodes the antigen-binding site of the antibody undergoes mutation at a high rate, resulting in the generation of variant Igs, some of which bind antigen with a higher affinity. This allows the affinity of the antibody response to increase. These mutations affect only somatic cells (B lymphocytes), and are not inherited through the germline.

Class-switch DNA recombination. The genetic process by which a B cell switches production of antibody or B cell receptor from one class to another, for example, from IgM to IgG, IgA, or IgE, results from an intrachromosomal deletional recombination between switch regions located upstream of exons of the constant heavy-chain (C_H) region. This exchanges the initially expressed C_H exons, such as Cμ, for an alternative set of downstream C_H exons, such as Cγ, Cα, or Cε, therefore, allowing the expression of antibodies that have the same antigen specificity but are of a secondary IgH isotype (IgG, IgA, or IgE) and thereby have a different biological effector function.

Features of Somatic Hypermutation and Class-Switch DNA Recombination

Antibody-mediated immune responses are critical to prevent invasion by pathogens, destroy cancer cells, or neutralize toxins. Antibodies or immunoglobulins (Igs) are produced by B cells or their differentiation elements, plasma cells. Antibody diversity and B cell development are underpinned by sequential Ig gene recombination (Casali 2014). This assembles noncontiguous Ig variable (V), diversity (D), and joining (J) genes into a functional V(D)J DNA segment, thereby producing the diverse pre-immune repertoire of B cell receptors (BCRs), B cell clonotypes, and corresponding antibodies. A BCR consists of the monomeric form of an Ig, which is identical to the secreted antibody (with exception of a transmembrane region instead of secreting region) and transmembrane proteins CD79a and CD79b. Pre-immune naive B cells express IgM with a low-to-moderate affinity for antigen. In response to antigen, selected IgM clones proliferate, differentiate, and undergo Ig gene somatic hypermutation (SHM) and class-switch DNA

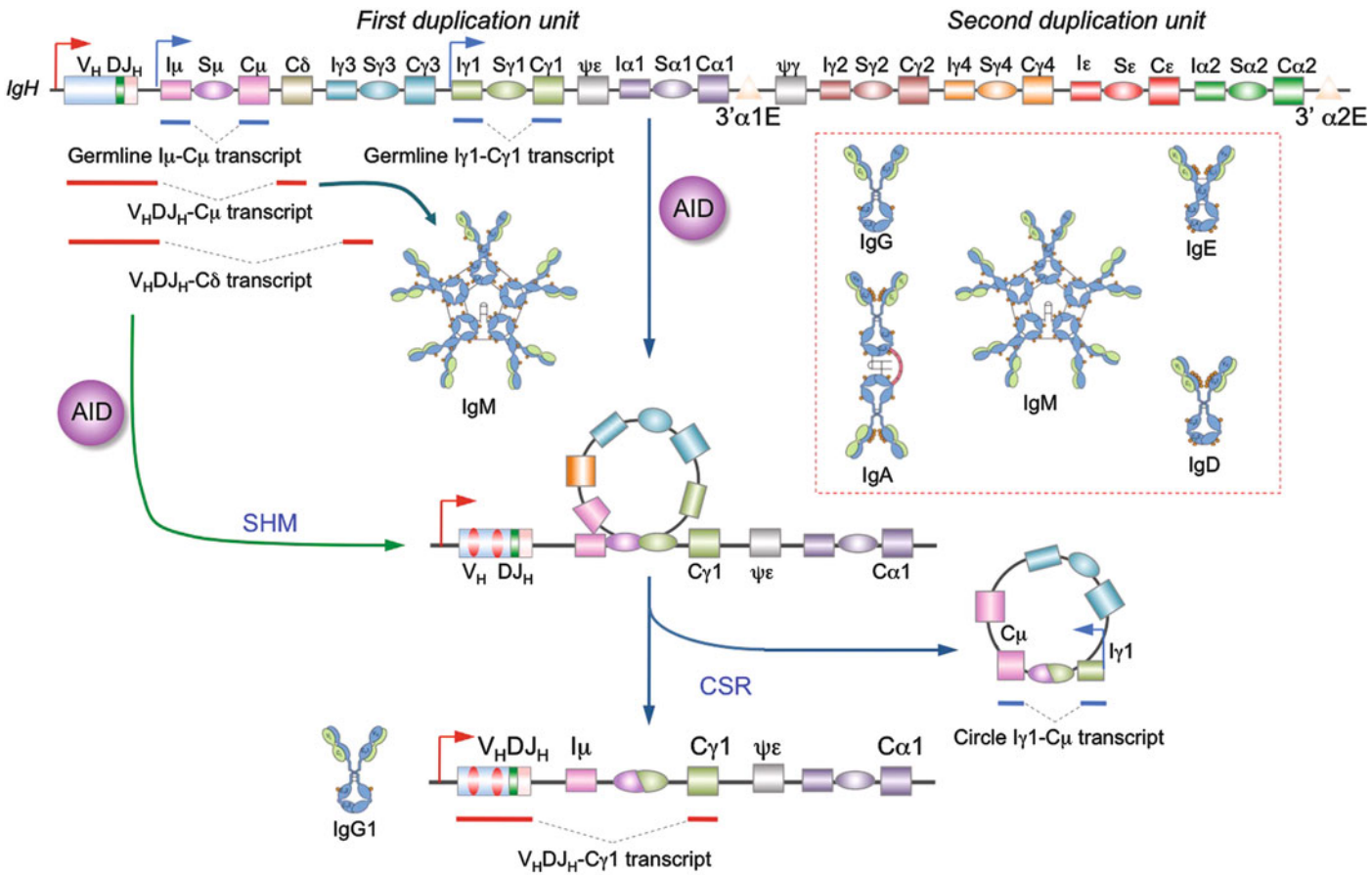
recombination (CSR). SHM and CSR are critical for the maturation of antibody responses to foreign and self-antigens. SHM targets the V(D)J portion of the antibody-encoding genes, thus providing the basis for positive selection by antigen of higher-affinity BCR mutants and, eventually, high-affinity antibodies (Casali et al. 2006). CSR replaces the constant (C)μ region of the heavy (H) chain with a downstream Cγ, Cα or Cε region, thereby providing antibodies with new biological effector functions.

SHM introduces mainly point mutations and, rarely, deletions or insertions into rearranged V(D)J gene sequences, but not C regions. The process is referred to as hypermutation because it introduces mutations at a rate that is a million-fold higher (10^{-3} change/base/cell division) than that of the spontaneous mutation rate in the genome at large (10^{-9} change/base/cell division). Random replacement mutations have unpredictable effects on protein function; some will decrease the affinity of the BCR for the antigen driving the response, whereas others will increase the intrinsic affinity for that antigen. B cells expressing a BCR with the highest affinity for antigen are positively selected and acquire a growth advantage over all other B cells; the other B cells are gradually counterselected for survival and proliferation. Further positive selection of the clone(s) that accumulated mutations conferring the highest affinity for antigen will result in narrowing clonal restriction and accumulation of high-affinity clones. SHM emerged before CSR in phylogeny, being fully functional in sharks, whose antibody responses show evidence of mutational selection. SHM is restricted to the Ig locus and a few other genes because abnormal and widespread mutations in the genome are detrimental to cell homeostasis and favor the emergence of neoplasia and autoimmunity.

CSR changes the antibody and BCR from one class to another by replacing the heavy-chain (IgH) C_H region with a different one, encoded by a downstream cluster of C_H exons through an intrachromosomal deletional DNA recombination. There are five antibody classes: IgM, IgD, IgG, IgA, and IgE, all with distinct tissue distribution and biological effector functions (Fig. 1) (Xu et al. 2012). IgM is the first BCR expressed and the first Ig produced by a differentiating B cell. IgM activates the complement efficiently. IgD is subsequently expressed as the B cell exits the bone marrow and acquires "mature" tracts. IgG comprises four subclasses (IgG1, IgG2, IgG3, and IgG4 in humans and IgG1, IgG2a, IgG2b, and IgG3 in mice) and is the most abundant Ig class in the circulation. While there is only one IgA class in mouse, IgA exists in two subclasses (IgA1 and IgA2) in human. IgMs are secreted as pentamers or hexamers, possess a high avidity for antigens with repetitive motifs (such as those occurring on most microbial pathogens), and activate complement efficiently. They, however, cannot pass into the extravascular space due to their large size. By contrast, monomeric IgG, monomeric IgE, and monomeric or dimeric IgA can distribute systemically to tissues where they mediate a variety of biological effector functions. While naïve B cells express only IgM and IgD, selected Ig classes and/or subclasses (isotypes) of antibodies are elicited during the course of an immune response, depending on the nature of the eliciting antigen and its entry mode. In humans, IgG1 and IgG3 are elicited mainly by viruses, IgG2 by encapsulated bacteria, IgG4 and IgE by large extracellular parasites, and IgA1 and IgA2 by pathogenic bacteria on mucosal surfaces. The constant regions of different immunoglobulin isotypes are encoded by different C_H exon clusters, which are organized in the order of Cμ, Cδ, Cγ, Cε, and Cα in the IgH locus. CSR results in the replacement of the expressed C_H exon cluster – for example, Cμ for IgM – with Cγ, Cα, or Cε, thereby giving rise to IgG, IgA, or IgE, respectively, in which the antigen-binding variable region is unaltered. Of note, IgD is generated not through CSR, but through alternative splicing of the primary transcripts that encode IgM.

DNA Lesions and Repair in SHM and CSR

Both SHM and CSR require transcription of their target DNA. CSR initiates with transcription

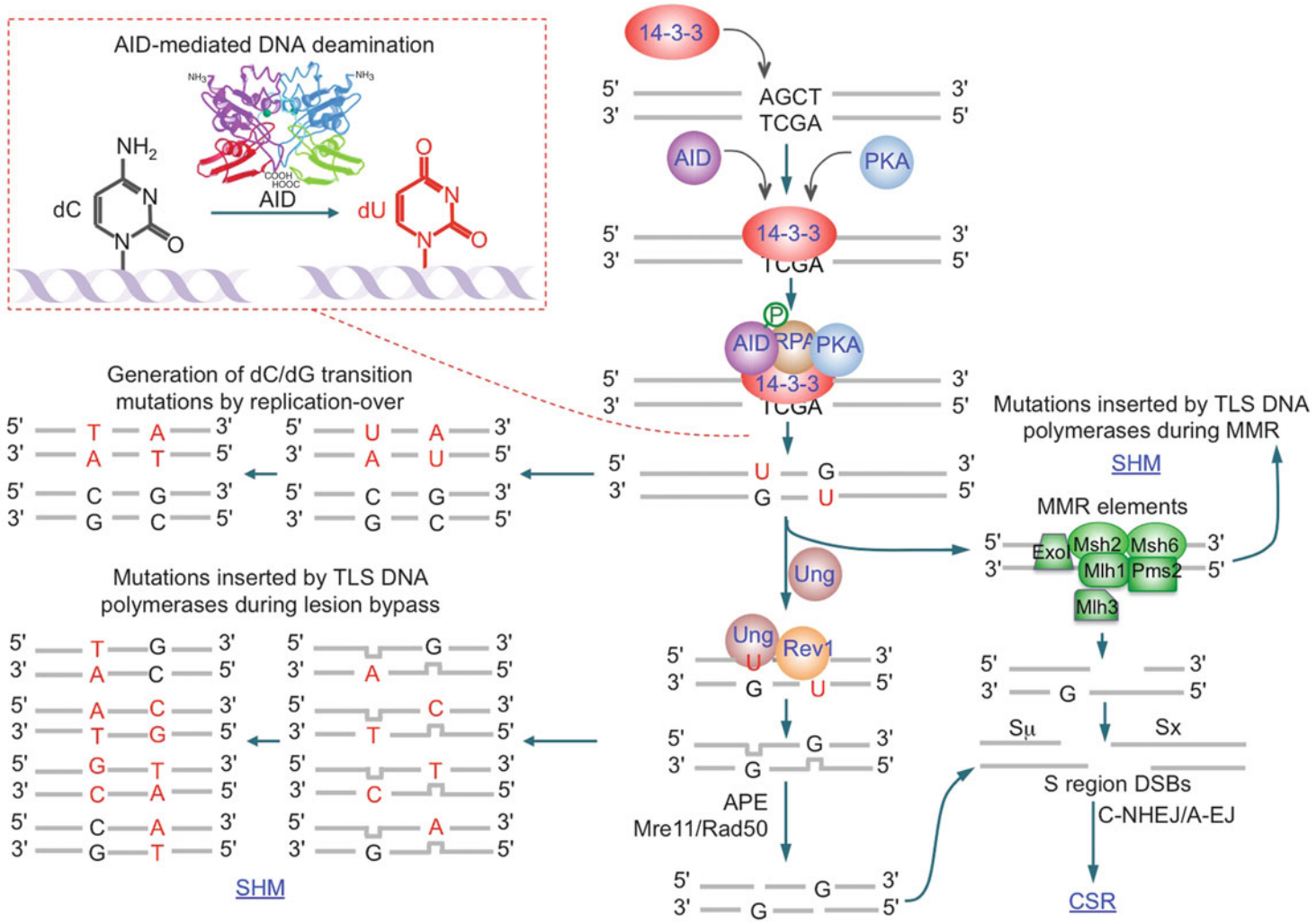


Immunoglobulin Somatic Hypermutation and Class-Switch DNA Recombination, Fig. 1 Schematic diagram of SHM and CSR. SHM introduces mainly point mutations in Ig V_HDJ_H, VκJκ, and VλJλ regions. There are five antibody classes (insert): IgM, IgD, IgG, IgA, and IgE, identified by the different heavy-chain constant (C_H) regions in their heavy chains (IgH). CSR exchanges the gene encoding the C_H region with one of a set of downstream C_H genes (depicted is CSR from Sμ to Sγ1 in the human *IgH* locus). Both SHM and CSR require AID. CSR involves the generation of DSBs in S regions (lying upstream of the constant-region gene) followed by DSB repair. This leads to juxtaposition of rearranged V_HDJ_H DNA with a downstream C_H exon cluster and deletion of the intervening sequence between S regions as an extrachromosomal circle

from the I_H promoters of the C_H regions that will be involved in the DNA recombination event. I_H promoters lie immediately upstream of all S (switch) regions. Such I_H promoters are activated upon binding of transcription factors induced by signaling of CD40, TLR, and receptors of cytokines, such as IL-4 or TGF-β. The I_H promoters that lie upstream of the upstream and downstream S regions that will be involved in the CSR event are activated to induce "germline" I_H–C_H transcripts, which are then spliced at the I_H region to join with the corresponding C_H region. SHM requires V_HDJ_H–C_H, Vκ–Cκ, or Vλ–Cλ transcription. In addition to both requiring transcription of the target DNA, SHM and CSR depend on activation-induced cytidine deaminase (AID)-mediated generation of DNA lesions and subsequent DNA repair (Casali et al. 2006; Muramatsu et al. 2000; Di Noia and Neuberger 2007). AID possesses structural similarities to the members of APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like), a class of enzymes that act on RNA to deaminate a cytidine to a uridine. AID, however, deaminates deoxycytosine (dC) residues in DNA to yield deoxyuridine (dU)/deoxyguanine (dG) mispairs. These mispairs then trigger DNA repair processes that lead to the introduction of mutations in V(D)J regions, or generation of double-strand DNA breaks (DSBs) in S regions and recombination of the DSB free ends (Fig. 1).

To mediate SHM and CSR, AID and other factors must be significantly upregulated and then targeted to the V(D)J regions where mutations will be introduced or the S regions that will undergo recombination. SHM preferentially targets the 5′-RGYW-3′ (R = A or G, Y = C or T, W = A or T) mutational hotspot. Interestingly, the 5′-AGCT-3′ iteration of 5′-RGYW-3′ is also a preferential target of DSBs in S regions. The dU generated by AID deamination of dC can be "replicated over" by pairing with deoxyadenine (dA) during replication. The emerging mutation is an obligatory dC → dT transition and a dG → dA transition on the complementary strand. The net result is the replacement of the original dC/dG pair with a dT/dA pair in half of the progeny cells. Alternatively, the dU can be removed from DNA by uracil DNA glycosylase (Ung) to give rise to an abasic site. Indeed, the key event in generating a random spectrum of mutations is the creation of an abasic site. This can be replicated over by an error-prone translesion DNA synthesis (TLS) polymerase, such as polymerase (pol) ζ, pol θ or pol η, each of which can insert all three possible mismatches (mutations) across the abasic site (Zan et al. 2001; Casali et al. 2006) or Rev1, which insert preferentially dC across an abasic site or other lesions. If the dC is not replicated over or the dU is not deglycosylated by Ung, the dU/dG mispair recruits the elements of mismatch repair machinery (MMR) machinery, starting with Msh2/Msh6, to excise the stretch of DNA containing the damage, thereby creating a gap that needs to be filled in by resynthesis of the missing DNA strand. This resynthesis is carried out by an error-prone TLS polymerase that will introduce mutations.

While the insertion of somatic mutations in V(D)J DNA requires only a single dC deamination, the generation of DSBs, which are obligatory CSR intermediates, entails processive AID-mediated deamination of dCs, particularly those within the 5′-AGCT-3′ tandem repeats in S regions, yielding high densities dUs in both DNA strands (Fig. 2). These are then processed by Ung, which is recruited to and stabilized on S regions by the scaffold functions of the translesion DNA polymerase Rev1 (Zan et al. 2012) and, possibly, 14-3-3 adaptors and replication protein A (RPA) (Xu et al. 2012), or elements of the MMR machinery, thereby leading to DSBs and/or point mutations. Rev1 is recruited to S region DNA in an AID-dependent fashion, interacts directly with Ung, and enhances Ung-mediated DNA dU glycosylation. Ung is a critical element of the base excision repair (BER) pathway. Excision of the abasic sites it creates by apurinic/apyrimidinic endonucleases (APEs) leads to single-strand DNA breaks (SSBs). Proximal SSBs in opposite strands would readily form staggered DSBs. In addition, key MMR elements such as MSH2 and MSH6 may be recruited to dU/dG mismatches adjacent to the SSB nicks, where they recruit 5′ → 3′ exonuclease I (Exo I) to yield DSBs.



Immunoglobulin Somatic Hypermutation and Class-Switch DNA Recombination, Fig. 2 (continued)

Staggered DSBs can also arise from AID- and Ung-dependent processing of blunt S region DSBs generated by endonuclease G (Zan et al. 2011). The DSB free ends upstream and downstream of the S region are joined by nonhomologous end joining (NHEJ) (Alt et al. 2013). In classic NHEJ (C-NHEJ), DSBs recruit Ku70 and Ku86 that form complexes with DNA-PKcs and play scaffold functions to then recruit other essential factors, such as the XRCC4–DNA ligase IV complex, to complete the end-joining process by the formation of S–S junctions. CSR can also occur, though, albeit at a lower efficiency, in the absence of XRCC4 or DNA ligase IV, through an alternative end-joining (A-EJ) pathway. The S–S junctions generated by A-EJ show higher microhomologies that those formed in C-NHEJ. A-EJ is also frequently associated with chromosomal translocations.

Induction of SHM and CSR

SHM and CSR are highly regulated, starting with their specific induction. AID is expressed late in the natural history of a B lymphocyte, after the B cell encounters antigen and differentiates in germinal centers of peripheral lymphoid organs, restricting SHM and CSR to this stage. In resting naïve or memory B cells, AID transcripts and protein are undetectable. These, however, are readily and greatly upregulated in B cells induced to undergo SHM and/or CSR by the same stimuli that trigger SHM/CSR.

AID expression in B cells is induced by T-dependent (TD) CD154 (CD40 ligand, CD40L)/CD40 engagement or T-independent (TI) TLR engagement by, mainly, microbe-associated molecular patterns (MAMPs), as synergized by BCR cross-linking (Xu et al. 2012; Pone et al. 2012). TI stimuli comprise TI type 1 (TI-1) and TI type 2 (TI-2). TI-1 stimuli generally include MAMPs, such as LPS or CpG, which activate their corresponding pattern recognition receptors (PRRs). The better-characterized PRRs include TLRs, NOD-like receptors (NLRs), RIG1-like receptors (RLRs), CARD helicases, C-type lectins, and scavenger receptors. TLRs, in particular, are highly expressed in B cells. In fact, B cells were known to respond to stimulation by MAMPs, such as LPS or flagellin, decades before the discovery of TLRs. TD and TI stimuli play a major role in the induction of AID and are, therefore, referred to as "primary" CSR- and, possibly, SHM-inducing stimuli. Stimulation of mouse B cells by LPS induces *Aicda* expression and germline Iγ3–S–Cγ3 transcription, leading to CSR to IgG3. In conjunction with cytokines IL-4, TGF-β, or IFN-γ, LPS induces CSR to all other isotypes. During B/T cell interactions, cytokines released by T cells accumulate in the

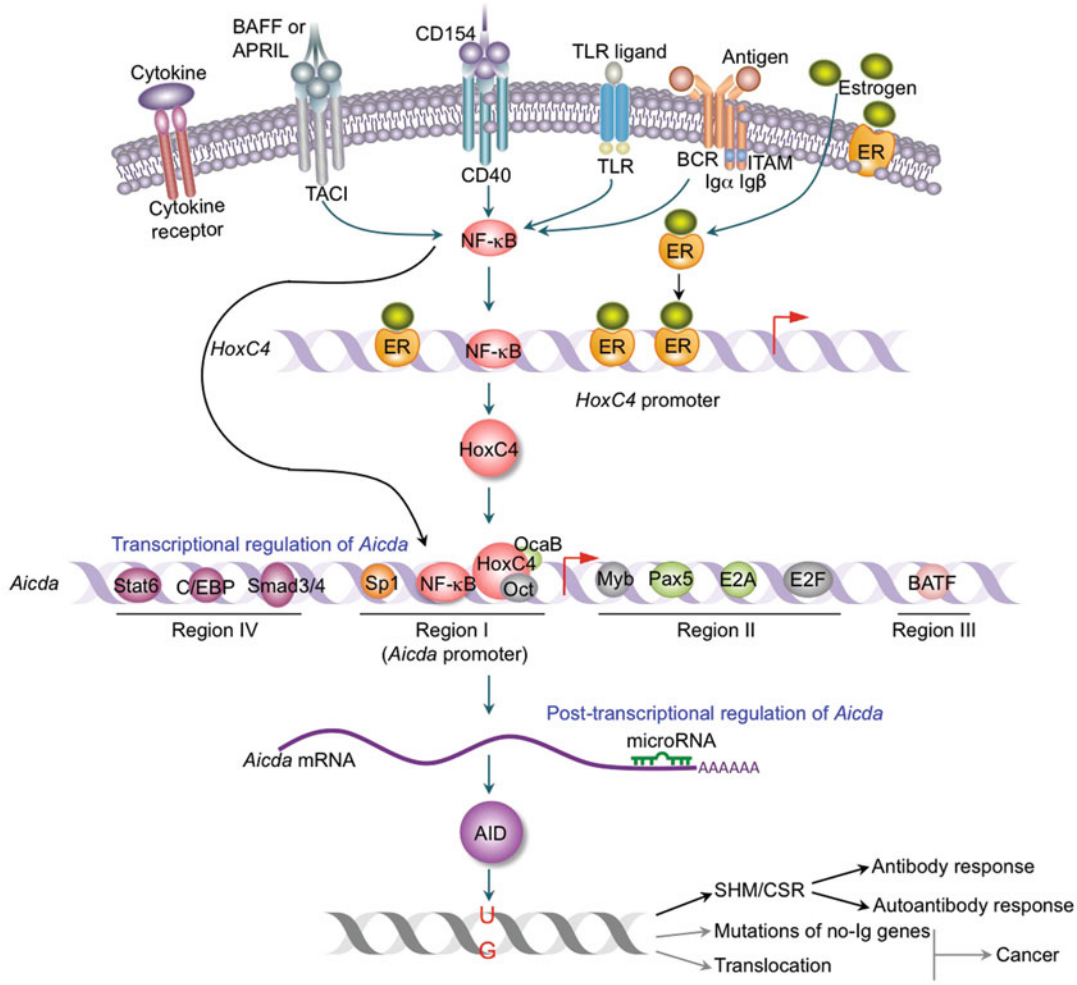
Immunoglobulin Somatic Hypermutation and Class-Switch DNA Recombination, Fig. 2 AID-mediated DNA lesions and lesion repair in SHM and CSR. Both SHM and CSR are initiated by AID-mediated dC deamination (insert). In SHM, the AID-mediated deamination of a dC base leads to the insertion of somatic mutation(s) in different ways. The dU/dG mispairs generated by AID in V(D)J regions can be replicated over and lead to dC to dT or dG to dA transition mutations. Excision of the dU residue by Ung gives rise to an abasic site, replication over which could yield either transition or transversion mutations at dC/dG pairs. The abasic site could also form a substrate for an APE, which can nick the DNA strand that contains the abasic site and allow polymerase-mediated repair by the conventional base excision repair (BER) pathway. Mutations at dA/dT pairs occur by an unidentified form of mutagenic patch repair that is triggered by recognition of the dU/dG mispair by MMR elements. This patch DNA repair process would recruit an error-prone TLS polymerase that introduces both transition and transversion mutations. In CSR, AID is targeted to S regions by 14-3-3 adaptors that specifically bind 5′-AGCT-3′ repeats in the S region core and recruit AID and PKA to S region DNA. AID also binds 14-3-3 proteins through its C-terminal region. RPA enhances AID deamination of dCs in transcribed S region DNA. The resulting dUs are removed by Ung, which is recruited and stabilized on S regions in a fashion dependent on the scaffold functions of Rev1, and possibly, RPA. Rev1 and RPA bind to different regions of Ung. Ung is also stabilized indirectly by AID within a putative macromolecular complex. Excision of Ung-generated abasic sites results in single-stranded breaks, which either directly form DSBs or are converted to DSBs in an Msh2-, Msh6-, and Exo I-dependent fashion. DSB resolution by C-NHEJ or A-EJ leads to formation of S–S junctions and CSR

space between the two cells and bind to their receptors on B cells. In addition to enhancing *Aicda* expression, as induced by primary stimuli, "secondary" CSR-inducing stimuli, i.e., cytokines (IL-4, TGF-β in humans, and IL-4, TGF-β, and IFN-γ in mice), direct CSR. "Primary" stimuli induce AID expression through activation of both the canonical and noncanonical NF-κB pathways. CD154 is expressed on the surface of activated T cells and engages its receptor CD40, which is constitutively expressed on the surface of B cells. CD40 is a member of the tumor necrosis factor receptor (TNF-R) superfamily, which includes other receptors such as B cell-activating factor receptor (BAFF-R) and BCMA (B cell maturation). Signals from activated TNF-Rs are first relayed to TNF-R-associated factors (TRAFs), which then cooperate with IκB kinases (IKKs) to activate the canonical NF-κB (e.g., TRAF6) or the noncanonical NF-κB (e.g., TRAF2/3 complex) pathway. Activated NF-κB heterodimers (canonical, p65/p50; noncanonical, p52/RelB) then translocate to the nucleus, where they synergize with HoxC4 and SP1/SP3 transcription factors to activate the *Aicda* promoter. Additional transcription factors, such as Stat6, C/EBP, Smad3/4, Myb, Pax5, E2A, E2f, and BATF, bind to other *Aicda* regulatory regions and can also play a role in regulation of *Aicda* gene expression.

"Secondary" stimuli induce germline (I_H–S–C_H) transcription through the S regions that are to undergo recombination, thereby specifying isotype selection. IL-4, which characterization of T helper type 2 (Th2) responses, directs B cells to switch to IgG1 and IgE, whose effector functions are tailored for the targeting and elimination of extracellular microbial pathogens and parasites. In the mouse, cytokines such as IFN-γ are secreted during T helper type 1 (Th1) responses, in which they stimulate the production of IgG2a, whose effector functions are suited for targeting and elimination of intracellular pathogens. TGF-β is abundant in mucosal tissues where it critically contributes to the induction of IgA. IgA antibodies control commensal microorganisms and pathogens in the intestine and the upper and lower respiratory tract. TNF-Rs BAFF-R, TACI, and BCMA are widely expressed on B cell surface where they are engaged by their ligands BAFF and APRIL. BAFF and APRIL were initially characterized as secreted TNF-R ligands that stimulate immature B cell proliferation to maintain homeostasis of peripheral B cells. They synergize with ligands of TLRs and BCR to induce enhanced *Aicda* expression and CSR.

Regulation of AID Expression and Activity

As a potent mutator, AID is under stringent transcriptional, posttranscriptional, and posttranslational regulation (Zan and Casali 2013). AID is also regulated in its targeting and enzymatic function. Transcription factors, such as HoxC4 and NF-κB, which are upregulated in a B cell lineage- and/or differentiation stage-specific manner, regulate the induction of AID (Park et al. 2009). HoxC4 acts in synergy with Oct1/Oct2 to induce AID expression by directly binding to the AID gene promoter through an evolutionarily conserved 5′-ATTTGAAT-3′ motif. HoxC4 is induced by the same stimuli that induce AID and CSR (Fig. 3). It is further upregulated by estrogen through three estrogen response elements (EREs) in its promoter region (Mai et al. 2010). *Aicda* transcription is also regulated by four major evolutionarily conserved regions in the AID gene locus through selected *cis*-regulating elements, many of which are binding sites of transcription factors that are activated by IL-4 and TGF-β. For example, signal transducer and activator of transcription 6 (Stat6), which is activated by IL-4, binds Region I located upstream of the promoter, and both Stat6 and TGF-β-activated Smad3 and Smad4 bind Region IV located 9 Kb upstream of the *Aicda* promoter. In addition, paired box protein 5 (Pax5) and E2A proteins bind Region II within the first intron, and the AP1 family transcription factor BATF binds Region III located 17 Kb downstream of the promoter. These transcription factors likely interplay with NF-κB, HoxC4, and Sp1/Sp3 at the promoter and enhancer elements, probably through long-range DNA interactions,



Immunoglobulin Somatic Hypermutation and Class-Switch DNA Recombination, Fig. 3 HoxC4 mediates estrogen-potentiated AID induction in antibody and autoantibody responses. T-dependent CSR-inducing stimuli (CD154) and T-independent CSR-inducing stimuli (namely, dual TLR–BCR, TACI–BCR, or TLR–TACI engagement) and cytokines induce HoxC4, mainly through activation of NF-κB. This induction is further enhanced by estrogen through binding of ERs to three evolutionarily conserved and cooperative EREs in the *HoxC4* promoter. By binding to a conserved HoxC4/Oct-binding site in the *Aicda* promoter, HoxC4 induces AID expression. In this function, HoxC4 acts in concert with transcription factors Oct1/Oct2 as well as Sp1/Sp3/NF-κB. The tissue and differentiation stage specificity of HoxC4 expression contributes to the tight regulation of AID expression. Estrogen enhances TLR- or CD154-mediated HoxC4 expression to induce SHM and CSR, and therefore, antibody and autoantibody responses. After transcription, the *Aicda* mRNA can be negatively regulated by microRNAs, including miR-155, miR-181b, miR-93, and miR-361, which bind to the conserved target sites on the 3′ UTR of *Aicda* mRNA

to induce or sustain *Aicda* expression by primary CSR-inducing stimuli and cytokines.

After transcription, the *Aicda* mRNA can be negatively regulated by microRNAs. A range of miRNAs are involved the regulation of immunity (Belver et al. 2011), including SHM and CSR. microRNAs are a family of single-strand, short (~22 nucleotides) noncoding RNAs that regulate gene expression in a sequence-specific manner. They bind to complementary sequences in the 3′-untranslational region (UTR) of target mRNAs, resulting in gene silencing by

translational repression or target mRNA degradation. microRNAs are abundantly present in all human cells, in which they modulate the expression of a few to hundreds of target genes each. miR-155, miR-181b, mir-361, and miR-93 regulate AID levels by binding to the evolutionarily conserved target sites in the 3′ UTR of *Aicda* mRNA, thereby reducing both *Aicda* mRNA and AID protein levels (Zan and Casali 2013; Li et al. 2013).

AID undergoes a series of posttranslational modifications, such as dimerization/oligomerization, nuclear/cytoplasmic translocation, phosphorylation, and polyubiquitination, all of which are important for AID activity. AID exists in both the nucleus and the cytoplasm of B cells (Zan and Casali 2013). It has been shown to undergo constant nucleocytoplasmic shuttling with a balance toward export to the cytoplasm. Although the molecular mechanism that governs the balance of AID nuclear import/export is unclear, it is conceivable that such a molecular mechanism provides B cells with two important functional features to (i) hamper the highly active and mutagenic AID from accessing DNA, thereby preventing indiscriminate dC deamination, DNA damage, and/or insertion of deleterious mutations, and (ii) generate a cytoplasmic AID reservoir, in which AID could be assembled with either SHM or CSR cofactors into a protein complex that, upon delivery of appropriate stimuli, can be promptly translocated into the nucleus and preferentially targeted to the *Ig* locus. The function of AID is likely also to be regulated in order to attain a balance between immunity and genomic instability, which would predispose to neoplastic transformation. The activity of AID is regulated by phosphorylation. Single-stranded DNA-binding protein RPA associates with phosphorylated AID from activated B cells and enhances AID activity on transcribed dsDNA containing SHM or CSR target sequences. RPA promotes the deamination of transcribed substrates by AID by stabilizing AID interaction with single-stranded DNA, which suggests that the role of RPA in SHM and CSR is to provide access of AID to target DNA. Further, the AID enzymatic activity, and therefore, CSR/SHM, can be inhibited by Fe^{2+}, which could displace Zn^{2+} in AID enzyme catalytic site by virtue of the similar chemical coordination properties of these two metal ions.

Targeting of the SHM and CSR Machinery

The targeting of critical SHM and CSR factors, especially, AID, is tightly controlled. As AID can introduce mutations in DNA, DSBs, and chromosomal translocations, it is tightly regulated not only in the expression and activity but also in its targeting. The specific targeting of the CSR machinery to the S regions that will undergo recombination relies on several factors (Xu et al. 2012). S regions contain a high frequency of 5′-AGCT-3′ repeats, accounting for more than 45 % of Sμ core DNA while for only about 1.3 % of DNA in the genome at large, including C_H regions. 5′-AGCT-3′ repeats are not only the preferred substrates for AID, they are also the specific targets of 14-3-3 adaptor proteins, which selectively target S region DNA and recruit AID, and possibly, other CSR factors, to mediate CSR (Xu et al. 2010). Because of the high density of 5′-AGCT-3′ repeats in all S regions, the inherent targeting of 5′-AGCT-3′ repeats by 14-3-3 adaptors alone cannot ensure the selective targeting by the CSR machinery of the upstream and downstream S regions that will undergo recombination, and not to the other S regions.

The S regions that are involved in CSR undergo high levels of transcription, indicating that they adopt an open chromatin state that allows for the recruitment of CSR factors (Stavnezer 2011). Single-stranded regions of DNA can be transiently exposed on the surface of RNA polymerase during transcription. The transcription-based mechanisms were also thought to provide AID with a single-strand DNA substrate, which is a preferential target of AID in vitro, in the context of a duplex substrate via looping out of the non-template strand. However, AID equally deaminates both substrate DNA strands during SHM and CSR.

The mechanism by which AID accesses the template DNA strand has been a major puzzle. Core RNA exosome complex has been shown to promote AID deamination of both template and non-template strands of in vitro transcribed SHM substrates. Integrity of the RNA exosome complex is required for optimal CSR. In B cells undergoing CSR, the RNA exosome complex associates with AID and accumulates on S regions in an AID-dependent manner, further suggesting that the RNA exosome plays an important role in targeting AID activity to both template and non-template strands of transcribed SHM/CSR targets. The recruitment, stabilization, and enzymatic function of AID require additional interactions, which involve the non-catalytic domains of these enzymes, nonenzymatic adaptors, such as 14-3-3 proteins, and selected histone modifications that are induced in the S region targets of CSR. In addition, CSR is impaired in B cells deficient in AID-binding proteins that also function in transcription or RNA processing. These proteins include Spt5 (AID binding to RNA polymerase II on transcribed S region DNA is likely mediated by Spt5) and Spt6, which regulate transcription; PTBP2, which regulates RNA splicing; RNA exosomes, which degrade RNA; the transcription-associated chromatin modifier; the facilitates chromatin transcription (FACT) complex components SSRP1 and Spt16; and RNA polymerase-associated factor (PAF) complex, which are involved in RNA processing, chromatin remodeling, exosome processing, and RNA pol II transcription elongation/pausing.

Targeting of the SHM and CSR machinery, including AID, also entails selected epigenetic modifications of the *Ig* locus, including the trimethylation of histone H3 lysine 4 (H3K4me3) and the combined acetylation of lysine 9 and phosphorylation of serine 10 in histone H3 (H3K9acS10ph) at the *IgH* locus, mainly in the S regions that are to undergo recombination (Li et al. 2013). These histone modifications not only reflect the open chromatin state of S regions that allows access of AID and other CSR factors but also provide additional specificity for DNA targeting by the CSR machinery. Indeed, H3K9acS10ph constitutes a specific chromatin target of 14-3-3 adaptors and plays a role in the recruitment/stabilization of 14-3-3 to S regions, and therefore, contributes to targeting of AID. SHM can occur only in VκJκ regions that exhibit DNA hypomethylation, suggesting that this epigenetic modification is critical for AID targeting in SHM. Indeed, methylated-CpG motifs have been found to be poor substrates for AID (Li et al. 2013). Further, the recruitment of AID to V(D)J regions can be facilitated by the germinal center-associated nuclear protein (GANP), which mediates histone modification and interacts with transcription elongation factors at V(D)J regions during SHM. One unique signature of hypermutating variable region is mammalian sterile kinase 1 (MST1)-mediated phosphorylation of histone H2B on serine 14 (H2BS14ph). In mammalian cells, phosphorylation of histone H2B is induced at late time points from DNA damage and accumulates in repair foci. It is likely that H2BSer14P plays a role in the recruitment or stabilization of components of the error-prone repair factors that resolve AID-dependent lesions. In addition, it has been shown in human cells that histone mark H3K36me3 is required to recruit the mismatch recognition protein MSH2/MSH6 onto chromatin through direct interactions with the MSH6 PWWP domain. It is possible that combinatorial signals generated through multiple histone modifications together with DNA demethylation orchestrate the execution of locus-specific mutagenesis.

Aberrant SHM and CSR and Diseases

Dysregulation of the SHM and CSR machineries is associated with variety of diseases (Zan and Casali 2013). The levels of CSR and SHM are critical in balancing efficient immunity with an autoimmune state. Lack of CD40, CD154, AID, or Ung results in hyper-IgM (HIGM) syndrome, a primary immune deficiency, in which CSR is reduced and SHM is reduced or altered, and therefore, loses the ability to make high-affinity IgG and IgA antibodies to combat bacterial

and viral infections. Deficiency of specific Ig isotype(s) is associated with some other primary immune deficiencies, such as "IgG subclass deficiency," "selective IgA deficiency," and "selective IgE deficiency." Aberrant CSR and/or SHM results in systemic or organ-specific autoimmunity, allergy, and asthma associated with atopic IgE, to neoplastic transformation.

Systemic lupus is an autoimmune disease characterized by the production of an array of pathogenic autoantibodies, including high-affinity anti-dsDNA IgG antibodies that are mutated and class switched, mainly to IgG, indicating that SHM and CSR are important in their generation. In addition, autoantibodies in which somatic mutations have been reverted to germline V regions showed low or no autoreactivity, further suggesting that anti-dsDNA autoantibodies develop from B cells reactive with self-antigen with low avidity or non-autoreactive B cells by SHM. In systemic lupus, the production of class-switched and hypermutated autoantibodies is associated with dysregulated AID expression, CSR, and SHM (Zan and Casali 2013).

Although the Ig loci constitute the major physiological targets of AID-mediated DNA deamination, yet other non-Ig locus DNA can also be targeted by AID, albeit at a much lower efficiency, especially under conditions of AID overexpression (Di Noia and Neuberger 2007), (Liu and Schatz 2009). A hallmark of mature B cell lymphomas is reciprocal chromosomal translocations involving the Ig locus and a proto-oncogene, which usually results in deregulated, constitutive expression of the translocated gene. In addition to such translocations, proto-oncogenes are frequently hypermutated in germinal center-derived B cell lymphomas. These chromosomal translocations and oncogene mutations play a critical role in the pathogenesis of most B cell lymphomas and are likely caused by aberrant, mis-targeted SHM and/or CSR (Klein and Dalla-Favera 2008). The tumorigenic ability AID was revealed in studies in AID transgenic mice. In addition, experiments with AID-deficient mice clearly showed that AID is required not only for the *c-myc*/*IgH* translocation but also for the malignant progression of translocation-bearing lymphoma precursor cells, probably by introducing additional genetic hits (Robbiani and Nussenzweig 2013). Normally, AID expression is only transiently and specifically induced in activated B cells in GCs. However, recent studies indicate that AID can be induced directly in B cells outside the GCs by various pathogens, including transforming viruses associated with human malignancies. Indeed, AID expression is not restricted to germinal center-derived B cell lymphomas but is also found in other types of B cell lymphoma and even in nonlymphoid tumors, suggesting that ectopically expressed AID is involved in tumorigenesis and disease progression in a wide variety of cell types.

Cross-References

- ▶ Autoantibodies in Rheumatoid Arthritis
- ▶ BCR Signaling
- ▶ CD40
- ▶ Epigenetics in Autoimmunity
- ▶ Micro-RNA in Autoimmunity
- ▶ NF-κB
- ▶ Systemic Lupus Erythematosus, Gender and Hormone Influences
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Alt FW, Zhang Y, Meng FL, Guo C, Schwer B. Mechanisms of programmed DNA lesions and genomic instability in the immune system. Cell. 2013;152:417–29.

Belver L, Papavasiliou FN, Ramiro AR. MicroRNA control of lymphocyte differentiation and function. Curr Opin Immunol. 2011;23:368–73.

Casali P. Somatic recombination and hypermutation in the immune system. In: Krebs JE, Goldstein ES, Kilpatrick ST, editors. Lewin's genes XI. Sudbury: Jones & Bartlett; 2014. p. 459–507.

Casali P, Pal Z, Xu Z, Zan H. DNA repair in antibody somatic hypermutation. Trends Immunol. 2006;27:313–21.

Di Noia JM, Neuberger MS. Molecular mechanisms of antibody somatic hypermutation. Annu Rev Biochem. 2007;76:1–22.
Klein U, Dalla-Favera R. Germinal centres: role in B-cell physiology and malignancy. Nat Rev Immunol. 2008;8:22–33.
Li G, Zan H, Xu Z, Casali P. Epigenetics of the antibody response. Trends Immunol. 2013;34:460–70.
Liu M, Schatz DG. Balancing AID and DNA repair during somatic hypermutation. Trends Immunol. 2009;30:173–81.
Mai T, Zan H, Zhang J, Hawkins JS, Xu Z, Casali P. Estrogen receptors bind to and activate the HOXC4/HoxC4 promoter to potentiate HoxC4-mediated activation-induced cytosine deaminase induction, immunoglobulin class switch DNA recombination, and somatic hypermutation. J Biol Chem. 2010;285:37797–810.
Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell. 2000;102:553–63.
Park SR, Zan H, Pal Z, Zhang J, Al-Qahtani A, Pone EJ, Xu Z, Mai T, Casali P. HoxC4 binds to the promoter of the cytidine deaminase AID gene to induce AID expression, class-switch DNA recombination and somatic hypermutation. Nat Immunol. 2009;10:540–50.
Pone EJ, Zhang J, Mai T, White CA, Li G, Sakakura JK, Patel PJ, Al-Qahtani A, Zan H, Xu Z, Casali P. BCR-signaling synergizes with TLR-signalling for induction of AID and immunoglobulin class-switching through the non-canonical NF-kappaB pathway. Nat Commun. 2012;3:767.
Robbiani DF, Nussenzweig MC. Chromosome translocation, B cell lymphoma, and activation-induced cytidine deaminase. Annu Rev Pathol. 2013;8:79–103.
Stavnezer J. Complex regulation and function of activation-induced cytidine deaminase. Trends Immunol. 2011;32:194–201.
Xu Z, Fulop Z, Wu G, Pone EJ, Zhang J, Mai T, Thomas LM, Al-Qahtani A, White CA, Park SR, et al. 14-3-3 adaptor proteins recruit AID to 5′-AGCT-3′-rich switch regions for class switch recombination. Nat Struct Mol Biol. 2010;17:1124–35.
Xu Z, Zan H, Pone EJ, Mai T, Casali P. Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. Nat Rev Immunol. 2012;12:517–31.
Zan H, Casali P. Regulation of *Aicda* expression and AID activity. Autoimmunity. 2013;46:83–101.
Zan H, Komori A, Li Z, Cerutti A, Schaffer A, Flajnik MF, Diaz M, Casali P. The translesion DNA polymerase zeta plays a major role in Ig and bcl-6 somatic hypermutation. Immunity. 2001;14:643–53.
Zan H, Zhang J, Al-Qahtani A, Pone EJ, White CA, Lee D, Yel L, Mai T, Casali P. Endonuclease G plays a role in immunoglobulin class switch DNA recombination by introducing double-strand breaks in switch regions. Mol Immunol. 2011;48:610–22.
Zan H, White CA, Thomas LM, Mai T, Li G, Xu Z, Zhang J, Casali P. Rev1 recruits ung to switch regions and enhances du glycosylation for immunoglobulin class switch DNA recombination. Cell Rep. 2012;2:1220–32.

Immunology of Alopecia in Autoimmune Skin Disease

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Synonyms

Autoimmune alopecia; Autoimmune hair loss; Discoid lupus; Discoid lupus erythematosus; DLE; Hair loss; Lichen planopilaris; LPP; Skin and alopecia; SLE; Systemic lupus erythematosus

Background, Definition, and Categorization of Alopecia

Hair loss can be divided into various categories (Baden 1987). Clinically, it is often divided into scarring and non-scarring forms of alopecia. It can also be divided by etiology, such as structural, genetic, nutritional deficiency, endocrine, and autoimmune. There is inexact overlap between these two approaches to categorization. For example, many, but not all, autoimmune forms of alopecia are also scarring. This entry focuses on autoimmune skin diseases that also cause hair loss. This includes alopecia from lichen planopilaris (LPP) a form of lichen planus, bullous diseases, discoid lupus erythematosus (DLE), and other forms of hair loss from connective tissue diseases like dermatomyositis, lupus, and scleroderma.

Structure and Function of the Hair Unit

In order to understand alopecia or hair follicle dysfunction, it is important to have a good understanding of the normal structure and function of hair follicles. Although derived from a single layer of multipotent embryonic ectoderm, the hair follicle is a complex, self-renewing structure (Dawber 1988; Muller et al. 1991). The hair follicles begin to form between embryonic days 75 and 80. Formation begins with dermal-epidermal signaling, initiating the formation of focal aggregates of basal epidermal cells, termed placodes or anlages. These first appear on the scalp and progress caudally. The placodes induce the underlying dermis to form primitive dermal papilla before extending down into the dermis itself to form, in combination with the early dermal papilla the bulbous peg hair. At this stage, the hair has two bulges. A superficial one which goes on to become the sebaceous gland and apparatus, and a deeper one which provides the insertion point for the erector pili muscle and the site for a persistent bulge wherein the hair follicle's stem cells are preserved. These points help define different regions of the hair. The infundibulum, superficial to the sebaceous gland, retains the structure of epidermis and keratinizes. Between the sebaceous gland and erector pili muscle lies the isthmus. In this region, there is no granular layer and keratinization does not occur. Below this is the stem or suprabulbar region, followed by the hair bulb or dermal papilla.

In the second trimester, hair follicles differentiate to form the seven concentric layers they will eventually recapitulate throughout their lifetime. From inside to out, these comprise: medulla, cortex, hair shaft cuticle, inner root sheath (Huxley's and Henley's layers and inner root sheath cuticle), and outer root sheath. The hair unit is completely formed at about 19–21-week gestation. At 24–28 weeks, the hairs leave anagen, the period of active growth, and enter catagen a phase of degeneration with resorption of the lower portion of the hair follicle and arrest of matrix cell division. While the epithelial cells at the base of the follicle undergo apoptosis, the dermal papilla itself remains intact and migrates upward, resting next to the follicular bulge. This new phase lasts days to weeks before transition to telogen, the hairs resting phase. Postnatally, the hairs continue to cycle through these phases, but asynchronously with about 80–90 % of hairs in anagen, 2 % in catagen, and 10 % in telogen at any one time. Anagen normally lasts about 7 years, catagen days to weeks, and telogen 3 months.

Immune Privilege and Inflammation of the Hair Follicle

Although perifollicular inflammation can occur in a variety of conditions, the end result can vary from no effect on the hair follicle, to a non-scarring alopecia, to a scarring alopecia (Harries et al. 2009). The difference between the latter two end-points seems to lie in the location of the inflammation with non-scarring alopecias such as alopecia areata having the majority of the inflammation around the bulb or stem of the hair follicle, the sections which normally apoptose or migrate, but subsequently regenerate (Alkhalifah et al. 2010). In contrast, scarring alopecias, like discoid lupus erythematosus (DLE) or lichen planopilaris (LPP), have predominant inflammation around the more superficial, permanent sections of hair wherein lie the stem cells and ostia (Al-Refu and Goodfield 2009). Destruction of the stem cells and fibrosis of the hair tract opening prevent hair follicle regeneration. However, given that certain non-scarring alopecias such as androgenetic alopecia and some non-alopecia inducing dermatitis also attract inflammatory cells to the isthmus, this cannot fully explain the different clinical outcomes.

One theory is that under normal circumstances, the stem cells are protected from immune damage, but in scarring alopecias, they lose their immune privilege. Indeed, studies support that both the anagen hair bulb (Christoph et al. 2000) and the bulge region (Meyer et al. 2008) of human hair follicles are immune privileged tissue. CD200 signaling is one potential means by which immune tolerance or loss of tolerance may be affected. CD200 is a type 1 transmembrane glycoprotein,

signaling of which via the CD200 receptor reduces inflammation and promotes tolerance (Rosenblum et al. 2006). In vitro experiments also show that antigen-presenting cell activity is downregulated by CD200 receptor activation, and CD200+ dendritic cells can inhibit activated T-cells from secreting pro-inflammatory cytokines (Barclay et al. 2002).

While this has not been proven as a mechanism in autoimmune skin disorders, the potential for it as a factor is supported by studies with CD200 knockout mice (Rosenblum et al. 2004). In these studies, skin from CD200-deficient (CD200−/−) mice grafted onto wild-type hosts (CD200+/+) developed inflammation, hair loss, and follicular scarring. In addition, the histopathology showed a peri- and intra-follicular mononuclear inflammatory cell infiltrate. This is likely highly pertinent to hair-pathology as in the bulge region of the hair follicle, CD200 transcripts are significantly upregulated (Ohyama 2007; Tumbar et al. 2004).

In addition to altered CD200 expression, immune suppression is evidenced by low-level MHC class I and ß2-microglobulin expression, as well as increased levels of immunosuppressants such as α-melanocyte-stimulating hormone, transforming growth factor-β1, indoleamine-2,3-dioxygenase, and macrophage migration inhibitory factor. In addition, gene expression profiling studies show upregulation of TGFβ2 mRNA and downregulation of INF-γ receptor expression and β2-microglobulin in bulge inflammation (Morris et al. 2004; Ohyama 2007; Tumbar et al. 2004). How this immune privilege is overcome in part appears to depend on the particular disease entity.

Connective Tissue Diseases: Alopecia of Discoid Lupus

The most well-recognized form of hair loss in connective tissue diseases is that of discoid (DLE) or chronic cutaneous lupus erythematosus (CCLE) which can occur in isolated DLE or as a manifestation of SLE. Scalp involvement occurs in 60 % of these patients, with up to 34 % of these patients experiencing permanent alopecia from inflammatory follicular destruction (Annessi et al. 1999; Fabbri et al. 2004; Trueb 2010; Wilson et al. 1992). DLE lesions are characterized by scaling, erythematous to violaceous papules with variable atrophy, follicular plugging, and telangiectasias and, in advanced lesions, scarring demonstrated by loss of follicular ostia (Fitzpatrick and Wolff 2008).

On histology, DLE lesions shows a superficial and deep, perivascular and periadnexal lymphocytic infiltrate with inflammation-induced interface vacuolar changes, and occasional apoptotic keratinocytes, at the dermal-epidermal junction of the hair follicles and interfollicular epidermis (McKee et al. 2005). Eosinophilic basement membrane zone thickening can be highlighted using special stains (e.g., periodic acid-Schiff [PAS]) and increased dermal mucin is usually present. Laminated keratin plugging of dilated follicular ostia may also be seen, as well as pigmentary incontinence with melanophages in the papillary dermis of older lesions. If performed, direct immunofluorescence studies will show, in 75 % of cases, dense granular deposition of immunoglobulin (most commonly IgG, but IgM and IgA may also be present) and complement factor C3 at the dermal-epidermal junction (Trueb 2010).

Apoptosis in DLE may in part be mediated via binding of the cell surface receptor, Fas (CD95) to its ligand, FasL (CD95L). In DLE, FasL is expressed on infiltrating macrophages and T-cells with a greater concentration of FasL+ macrophages around the hair follicle. Fas itself is upregulated on epidermal keratinocytes, leading to rapid keratinocyte apoptosis (McNally et al. 1997; Nagafuchi et al. 2002; Nakajima et al. 1997). An alternative apoptotic mechanism is via the p53 pathway. p53 allows cell cycle arrest for DNA repair or, when DNA damage is too extensive, can induce cellular apoptosis and is aberrantly expressed in lupus and dermatomyositis (Miret et al. 2003; Pablos et al. 1999) and Bcl-1, which can block cellular apoptosis, is downregulated in the epidermis of DLE lesions. In addition, mice lacking the p53 effector gene Gadd45a develop a lupus-like syndrome (Salvador et al. 2002).

Evidence of this damage to the bulge region, wherein lie the stem cells, is provided by keratin-15

(Al-Refu and Goodfield 2009). Normally, Keratin-15 immunostaining shows upregulation in the bulge region of the outer root sheath (ORS). However, it is reduced in the bulge region of DLE lesions. This may reflect either the irreversible apoptotic damage noted above or that these cells have been induced to differentiate potentially losing their stem cell potential.

As noted above, there is often a significant upregulation of mucin or dermal glycosaminoglycans in DLE lesions. It has been shown that such glycosaminoglycans themselves also possess hair growth- inhibitory properties (Paus 1991).

Connective Tissue Diseases: Other Forms of Alopecia

Although DLE-like lesions may be the most widely recognized and, given its scarring nature, potentially the most serious form of SLE-associated alopecia, it is not the most common form of hair loss in systemic lupus patients. Indeed, in one study, it was reported that of 122 lupus patients, 104 (85.2 %) experienced some form of hair loss, with the most common being a diffuse non-scarring alopecia that occurred in 65.1 % of patients (Yun et al. 2007). This latter, diffuse form of hair loss also occurs in other connective tissue diseases, such as scleroderma and dermatomyositis. The pathogenesis of this form of hair loss is poorly understood. In part, it is likely due to a chronic telogen effluvium, the persistence of which ebbs and flows with the flares in the underlying connective tissue disease, but just as in other forms of telogen effluvium, may lag by up to 3–6 months in its clinical manifestation, making diagnosis and association problematic.

Another clinically recognizable entity is the presence of "lupus hairs" which consist of diminution of the hairs at the peripheral hairline. Most notably cosmetically this results in the appearance of a step back of the anterior hairline. It has been hypothesized that this results from an interruption of the normal hair growth cycle from the induction of a negative nitrogen balance (Trueb 2010). As a result, the hairs become fragile and easily fragment above the skin surface. In its most extreme form, patients experience a dystrophic anagen effluvium. In this situation, severe flares of the underlying illness can result in a temporary shutdown of hair production at the dermal papillae. As in chemotherapy or analogous to Beau's lines in the fingernail, this produces a stricture in the hair shaft (Pohl–Pinkus constriction) that can fracture either intra- or extra-follicularly.

There is also reported a patchy alopecia of SLE which occurs in up to 15 % of patients. This clinical entity is very poorly circumscribed, and it remains debatable whether it really exists, or if it may mimic other forms of alopecia and be somewhat overdiagnosed. In some studies, it is described as a mild erythema but without scarring, leading to the possibility that these are merely mild manifestations of DLE. In other studies, it is reported that almost all of the remaining hairs with such patches are telogen hairs, or dystrophic anagen hairs. Finally, some studies report that on histopathology, a peribulbar infiltrate of lymphoid cells is found surrounding miniaturized anagen hair bulbs and fibrous streamers below telogen hairs. This raises the question as to whether in these cases, this is an incipient form of alopecia areata, a separate autoimmune disease of the hair follicle which can be associated with lupus (Werth et al. 1992).

As in DLE, SLE and other connective tissue diseases also have an upregulation of dermal mucin which has itself anti-proliferative effects on the hair follicle. In the most extreme case of upregulated mucin, this can go beyond mere cytokine signaling and actual invasion of the hair follicle with follicular mucinosis occurring, leading to a scarring destruction of the hair follicle.

Finally, in many patients, the medications used to treat their lupus, such as plaquenil or methotrexate, can themselves exacerbate hair loss problems and should be carefully reviewed in any patient experiencing this problem. A simple test is to reduce the dose of the medication in question. If the hair loss is lupus related, the lupus will worsen and the hair loss increase on the lower dosage. In patients with hair loss from the medication however, provided the lupus itself remains well controlled, the hair loss in these cases will diminish.

Lichen Planopilaris

Lichen planopilaris (LPP) is histologically the follicular form of the inflammatory skin disorder lichen planus (Assouly and Reygagne 2009). They may occur together or separately. Although rare, LPP is the most frequent cause of adult primary scarring alopecia. The disorder is believed to be autoimmune in etiology, in part because of characteristic immunohistology, although no specific serologic markers are known. As with many autoimmune disorders, LPP is more common in women. The typical age of onset is between 40 and 60 years.

Despite a histopathological similarity, clinically LP and LPP are quite distinct and the purple, polygonal flat topped papules of LP are not observed. Instead, in the affected areas, there is perifollicular erythema, acuminate keratotic plugs, and on occasion bigeminate hairs. In these early stages, patients complain of itching, pain, or burning of the scalp. In contrast to DLE lesions, there is no dischromy, telangiectasia, or scale. LPP is usually subdivided into three variants: classic LPP, frontal fibrosing alopecia (FFA), which presents as a progressive symmetric band-like alopecia around the anterior and lateral hairlines, including on occasion the retroauricular areas, and Lassueur Graham-Little Piccardi syndrome, which consists of scarring patchy alopecia of the scalp, noncicatricial axillary and pubic hair loss, and a lichenoid follicular eruption. Some authors add a fourth category, namely, fibrosing alopecia in a pattern distribution (FAPD), which in most instances is clinically similar to the pattern of androgenetic alopecia, but on histopathology, fibrosis is seen.

On histology, LP is characterized by a lichenoid interface inflammation with hypergranulosis, hyperkeratosis, hyperacanthosis, degeneration of basal keratinocytes, and occasional destruction of the basal layer (McKee et al. 2005). Colloid bodies, degenerated keratinocytes that stain deeply eosinophilic, are often seen in the basal layer. In early LPP, these same changes occur around hair follicles most prominently between the infundibulum and the isthmus, with sparing of the stem and dermal papilla. In late-stage lesions, inflammation is minimal and the hair follicles are replaced with fibrous tracts. In 40 % of cases, the DIF can show positive staining for IgM, or less commonly IgA or C3, with a linear band of fibrin or fibrinogen at the dermo-epidermal junction.

While there is debate regarding the mechanism by which LPP causes hair follicle destruction, as in DLE, it is observed that in LPP, keratin-15 is reduced in the bulge region of affected follicles, suggesting that targeting of the bulge region is important (Mobini et al. 2005), and involves either destruction or forced differentiation of the stem cells therein. In addition, bulge cells in LPP-affected follicles show reduced proliferation rates when compared with bulge cells from un-involved follicles.

However, it has also been demonstrated that LPP follicles also exhibit a downregulation of the gene, PPAR-γ. This results in accumulation of lipids within the follicle and abnormal sebum production. This may be relevant as production of a PPAR-γ knockout mouse was shown to recapitulate the lymphocytic, cicatricial LPP phenotype, thus suggesting an alternative pathogenesis and treatment target (Billoni et al. 2000; Karnik et al. 2009).

Bullous Diseases

As noted above, the infundibular portion of the hair follicle is continuous with, and biochemically similar to, the interfollicular epidermis. This includes the basement membrane zone with expression of plectin, 180-kD bullous pemphigoid antigen (BP180), 230-kD bullous pemphigoid antigen (PB230), a6b4 integrin, laminin-311, laminin-332, and type 4 and type 7 collagen. There is also some expression of these factors into the isthmus, where the stem cell bulge exists. All of these are potential targets of autoimmune attack and in the skin produce characteristic bullous diseases (McKee et al. 2005; Miteva et al. 2011; Rosenblum et al. 2004). For example, anti-desmoglein-3 antibodies produce pemphigus vulgaris with blistering of the skin and mucosal surfaces. Given the presence and importance of these proteins in the hair unit, it would be reasonable to expect a similar

end-stage scarring alopecia in these conditions as is found in the foregoing conditions.

However, despite the observation that scalp involvement is common in such bullous diseases as pemphigus vulgaris (PV) and pemphigus foliaceus (PF), in humans, hair loss is rarely reported. A variety of explanations for this are possible. For one the distribution of the proteins may make attack of the stem cell bulge less likely. For example, although in the infundibulum, as in the interfollicular epidermis, desmoglein-3 is present predominantly in the basal cells, it is reduced in the isthmus where the stem cells occur. Alternatively, it may be that the immune privilege collapse of other conditions does not occur in these conditions in humans. Further studies, especially in comparison to animal models such as dogs, cats, goats, and horses, where alopecia does result from these same bullous diseases, are important, to elucidate these differences.

Cross-References

- ▶ Alopecia Areata
- ▶ Discoid SLE
- ▶ Lichen Planus

References

Alkhalifah A, et al. Alopecia areata update: part I. Clinical picture, histopathology, and pathogenesis. J Am Acad Dermatol. 2010;62(2):177–88. quiz 189–90.

Al-Refu K, Goodfield M. Hair follicle stem cells in the pathogenesis of the scarring process in cutaneous lupus erythematosus. Autoimmun Rev. 2009;8(6):474–7.

Annessi G, et al. A clinicopathologic study of scarring alopecia due to lichen planus: comparison with scarring alopecia in discoid lupus erythematosus and pseudopelade. Am J Dermatopathol. 1999;21(4):324–31.

Assouly P, Reygagne P. Lichen planopilaris: update on diagnosis and treatment. Semin Cutan Med Surg. 2009;28(1):3–10.

Baden HP. Diseases of the hair and nails. Chicago: Year Book Medical; 1987.

Barclay AN, et al. CD200 and membrane protein interactions in the control of myeloid cells. Trends Immunol. 2002;23(6):285–90.

Billoni N, et al. Expression of peroxisome proliferator activated receptors (PPARs) in human hair follicles and PPAR alpha involvement in hair growth. Acta Derm Venereol. 2000;80(5):329–34.

Christoph T, et al. The human hair follicle immune system: cellular composition and immune privilege. Br J Dermatol. 2000;142(5):862–73.

Dawber RP. The embryology and development of human scalp hair. Clin Dermatol. 1988;6(4):1–6.

Fabbri P, et al. Scarring alopecia in discoid lupus erythematosus: a clinical, histopathologic and immunopathologic study. Lupus. 2004;13(6):455–62.

Fitzpatrick TB, Wolff K. Fitzpatrick's dermatology in general medicine. 2 vols. New York: McGraw-Hill Medical; 2008.

Harries MJ, Meyer KC, Paus R. Hair loss as a result of cutaneous autoimmunity: frontiers in the immunopathogenesis of primary cicatricial alopecia. Autoimmun Rev. 2009;8(6):478–83.

Karnik P, et al. Hair follicle stem cell-specific PPARgamma deletion causes scarring alopecia. J Invest Dermatol. 2009;129(5):1243–57.

McKee PH, Calonje E, Granter SR. In: McKee PH, Calonje E, Granter SR, editors. Pathology of the skin: with clinical correlations. Edinburgh/Philadelphia: Elsevier Mosby; 2005.

McNally J, et al. Fas ligand expression and function in systemic lupus erythematosus. J Immunol. 1997;159(9):4628–36.

Meyer KC, et al. Evidence that the bulge region is a site of relative immune privilege in human hair follicles. Br J Dermatol. 2008;159(5):1077–85.

Miret C, et al. Relationship of p53 with other oncogenes, cytokines and systemic lupus erythematosus activity. Tumour Biol. 2003;24(4):185–8.

Miteva M, Murrell DF, Tosti A. Hair loss in autoimmune cutaneous bullous disorders. Dermatol Clin. 2011;29(3):503–9, xi.

Mobini N, Tam S, Kamino H. Possible role of the bulge region in the pathogenesis of inflammatory scarring alopecia: lichen planopilaris as the prototype. J Cutan Pathol. 2005;32(10):675–9.

Morris RJ, et al. Capturing and profiling adult hair follicle stem cells. Nat Biotechnol. 2004;22(4):411–7.

Muller M, et al. Embryology of the hair follicle. Early Hum Dev. 1991;26(3):159–66.

Nagafuchi H, et al. Aberrant expression of Fas ligand on anti-DNA autoantibody secreting B lymphocytes in patients with systemic lupus erythematosus: "immune privilege"-like state of the autoreactive B cells. Clin Exp Rheumatol. 2002;20(5):625–31.

Nakajima M, et al. Expression of Fas ligand and its receptor in cutaneous lupus: implication in tissue injury. Clin Immunol Immunopathol. 1997;83(3):223–9.

Ohyama M. William J. Cunliffe Scientific Awards. Advances in the study of stem-cell-enriched hair follicle bulge cells: a review featuring characterization and isolation of human bulge cells. Dermatology. 2007;214(4):342–51.

Pablos JL, et al. Keratinocyte apoptosis and p53 expression in cutaneous lupus and dermatomyositis. J Pathol. 1999;188(1):63–8.
Paus R. Hair growth inhibition by heparin in mice: a model system for studying the modulation of epithelial cell growth by glycosaminoglycans? Br J Dermatol. 1991;124(5):415–22.
Rosenblum MD, et al. Expression of CD200 on epithelial 'cells of the murine hair follicle: a role in tissue-specific immune tolerance? J Invest Dermatol. 2004;123(5):880–7.
Rosenblum MD, et al. CD200, a "no danger" signal for hair follicles. J Dermatol Sci. 2006;41(3):165–74.
Salvador JM, et al. Mice lacking the p53-effector gene Gadd45a develop a lupus-like syndrome. Immunity. 2002;16(4):499–508.
Trueb RM. Involvement of scalp and nails in lupus erythematosus. Lupus. 2010;19(9):1078–86.
Tumbar T, et al. Defining the epithelial stem cell niche in skin. Science. 2004;303(5656):359–63.
Werth VP, et al. Incidence of alopecia areata in lupus erythematosus. Arch Dermatol. 1992;128(3):368–71.
Wilson CL, et al. Scarring alopecia in discoid lupus erythematosus. Br J Dermatol. 1992;126(4):307–14.
Yun SJ, et al. Cross-sectional study of hair loss patterns in 122 Korean systemic lupus erythematosus patients: a frequent finding of non-scarring patch alopecia. J Dermatol. 2007;34(7):451–5.

Immunosuppression in Clinical Liver Transplantation

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Synonyms

Immunosuppressive therapy; Liver failure; Solid organ transplantation

Definitions

Liver transplantation is an established therapy for people with acute or chronic liver failure. In addition, patients with unresectable hepatocellular carcinoma may require liver transplantation. As the new liver is recognized and rejected by the immune system as foreign, patients need to be treated with agents hampering the immune system.

Introduction

Liver transplantation is the last therapeutic option for patients with acute liver failure or end-stage hepatic disease. Although 1-year graft survival is excellent and currently exceeds 85 %, side effects of the immunosuppressive treatment and recurrence of the underlying liver disease (hepatocellular carcinoma, autoimmune hepatitis, hepatitis C virus infection, etc.) remain major issues in liver transplantation. With approximately 80 % of liver transplant recipients treated, calcineurin inhibitors (CNI) remain the cornerstone of immunosuppression. However, CNI are nephrotoxic and have been suggested to be pro-cancerous and therefore should be avoided in patients with renal impairment or hepatocellular carcinoma. Steroids are often used in addition to CNI but have severe metabolic side effects when used long term. In addition, patients infected with hepatitis C virus (HCV) rapidly progress to cirrhosis within a few years after transplantation, in particular when treated with steroids. Hence, immunosuppressive treatment needs to be adjusted to patients' conditions and underlying diseases. In contrast to the surgical procedures, which are highly standardized, the variety of immunosuppressive agents and possible drug combinations have resulted in center-specific protocols. Current strategies are trying to choose the most suitable, well-tolerated combination of drugs for each patient.

Historical Background

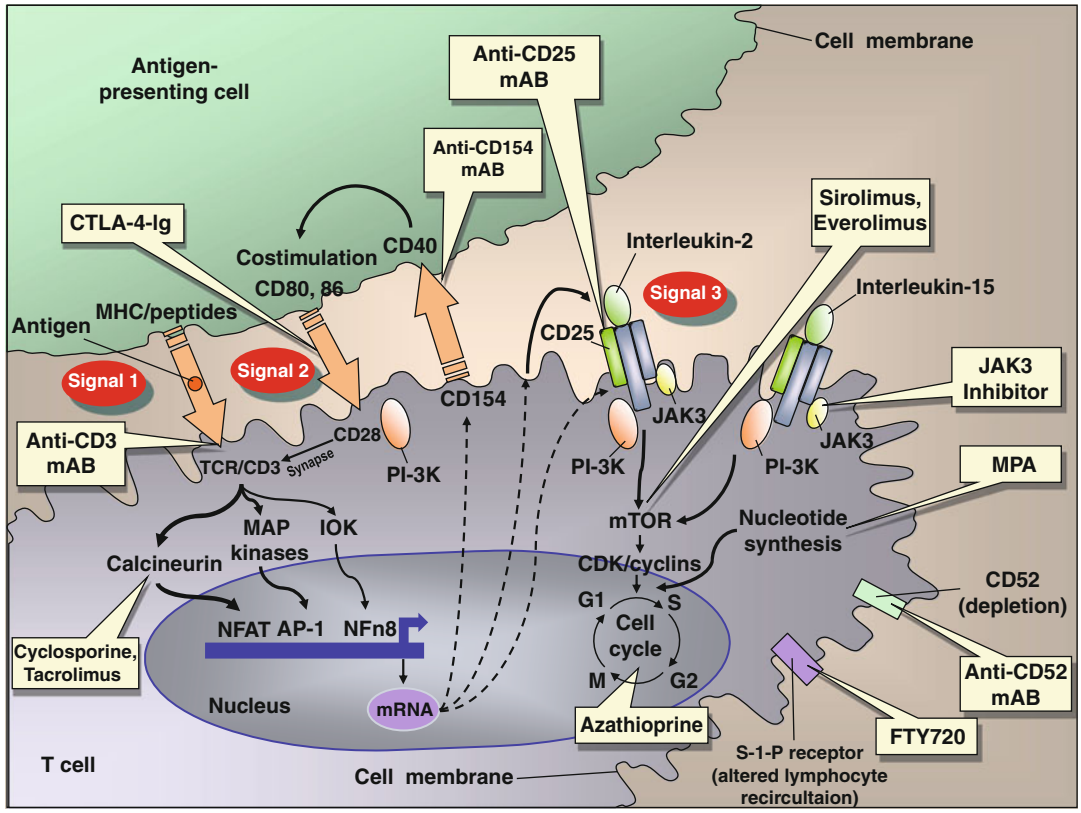
Besides surgical issues, a major hurdle in the beginning of solid organ transplantation was acute rejection of the transplanted organ by the

host immune system. For a long time, whole body irradiation was the only available method to induce immunosuppression. In the 1960s, the first pharmaceutical breakthrough was achieved with the antimetabolite of purine synthesis 6-mercaptopurine and later its less toxic derivate azathioprine (Aza) and glucocorticosteroids (GS). In 1963, Starzl and Murray could show an extension of patient and graft survival using this combination in the kidney transplant setting. After the introduction of antilymphocyte sera (ALS), which had shown immunosuppressive effects in canine models of kidney and liver transplantation, Starzl reported in 1968 the survival of a 1½-year-old girl for more than 13 months following allogeneic liver transplantation using Aza, GS, and ALS. Following this achievement, liver transplant programs were established in several centers around the world, but 1-year survival remained poor ranging from 30 % to 35 %. In 1976, Borel described the immunosuppressive effects of cyclosporin A (CsA), which revolutionized the field of immunosuppressive treatment. Sir Roy Calne reported in 1979 his encouraging results obtained with CsA in renal and liver transplant recipients, which led to the approval of liver transplantation for the treatment of end-stage liver disease in 1983 by a consensus meeting at the National Institutes of Health. In 1994, tacrolimus (Tac), another calcineurin inhibitor, was approved by the FDA for liver transplant recipients. In the early 1990s, Sollinger introduced mycophenolate acid (MPA), a cell cycle inhibitor, in renal transplant recipients, which was subsequently used instead of Aza and later even as monotherapy. In 1999, sirolimus, an mTOR inhibitor, was approved for renal transplant recipients by the US *Food and Drug Administration* (FDA) and raised great interest as besides its immunosuppressive function, sirolimus showed anticancerous and antifibrotic effects. Since then, several new agents have been used in liver transplant recipients, e.g., the IL-2 receptor-blocking antibody basiliximab. However, neither sirolimus nor basiliximab have been approved for treating liver transplant recipients yet.

Immunosuppressive Agents

Often, a combination of different immunosuppressive agents is used in recipients of solid organ transplantation. Figure 1 (adopted from (Post et al. 2005)) shows the molecular mechanism of some of the most often used drugs in liver transplantation, which are described in detail below.

Glucocorticosteroids (*GC*) are used since the beginning of solid organ transplantation and remain an important part of immunosuppression in liver transplant recipients. GC are hormones synthesized from cholesterol in the adrenal glands, and GC have been shown to induce apoptosis of immature T cells and stimulate T cells to home to lymphoid tissue. GC also suppress both B-cell clone expansion and antibody production, and they have an effect on dendritic cells (DC), where they are thought to induce a tolerogenic phenotype. In addition, GC downregulate the expression of Fc receptors on macrophages, thereby reducing their capacity to phagocytose opsonized cells. On a molecular level, GC bind to the intracellular cytosolic glucocorticoid receptor, which can induce three different actions all of which are thought to contribute to the anti-inflammatory action of GC. Upon activation, the glucocorticoid receptor can translocate to the nucleus, where it either induces the transcription of noninflammatory proteins or inhibits the transcription factors lAP-1 and NF-kB, which are important for the transcription of proinflammatory cytokines like interleukin (IL)-1, IL-2, IL-6, and interferon-gamma (IFN-γ). In addition to these two genomic effects, GC can block T-cell activation by interfering with a T-cell receptor signaling complex formed by the glucocorticoid receptor, *Heat Shock Protein 90* (HSP90), *lymphocyte-specific protein kinase* (LCK), and the tyrosine kinase FYN. GC also induce lipocortin-1 synthesis, which binds to the cell membrane and prevents phospholipase A2 from coming into contact with arachidonic acid leading to less eicosanoids. Lipocortin-1 also binds to leukocyte membrane receptors and inhibits epithelial adhesion,



Immunosuppression in Clinical Liver Transplantation, Fig. 1 Molecular mechanism of immunosuppressive agents used in liver transplantation (Adopted from Post et al. 2005)

emigration, chemotaxis, phagocytosis, respiratory burst, and the release of cytokines, chemokines, and lysosomal enzymes from neutrophils and macrophages. GS have several side effects which include hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis, cataract, and hypertension.

Calcineurin inhibitors (*CNI*) revolutionized solid organ transplantation and increased the 1-year survival rates of liver transplant recipients to 85 %. Two different CNI are currently used: *cyclosporin A* (CsA) that binds to cyclophilin and *tacrolimus* (Tac), which binds to *FK-binding protein 12* (FKB12), both leading to inhibition of calcineurin, a calcium-/calmodulin-dependent serine/threonine phosphatase. Although Tac inhibits calcineurin 100-fold more potent than CsA, the same appears to be true for their side effects.

Calcineurin dephosphorylates *nuclear factor of activated T cells* (NF-AT), a lymphocyte-specific cytoplasmatic-based transcription factor, which translocates to the nucleus and binds to the promoter regions of cytokines including IL-2, IL-3, IL-4, granulocyte-macrophage colony-stimulating factor, IFN-γ, and tumor necrosis factor alpha (TNF-α). Therefore, CNI primarily target the activation and proliferation of naïve T cells and the function of effector T cells. A major side effect of CNI is renal toxicity leading to severe tubular atrophy, interstitial fibrosis, and focal hyalinosis of small arteries and arterioles. Nephrotoxicity has been linked to the inhibition of calcineurin, but animal data suggests that the inhibition of cyclophilin A may be responsible for nephrotoxicity of CNI. There is no difference in the nephrotoxicity of Tac or CsA.

Cyclosporin A (*CsA*) is a cyclic polypeptide comprised of 11 amino acids which was discovered as a product by the soil fungus Tolypocladium inflatum in 1972. CsA was

initially released as Sandimmune® (Novartis) and required emulsification prior to intake due to poor solubility in water. However, this formulation was associated with high variations in bioavailability ranging from 1 % to 89 % with a mean of 30 %. Consequently, a newer formulation, Neoral® (Novartis), using microemulsion preconcentrates with a drug in a lipophilic solvent, was released leading to more consistent CsA trough levels. In addition to typical side effects of CNI, cyclosporin A also seems to induce cancer progression, a mechanism, which appeared to be dependent on tumor growth factor beta (TGF-β) (Hojo et al. 1999).

Tacrolimus (*Tac*)*/FK506*, a macrolide compound, was discovered by Fujisawa Pharmaceutical Company in 1984 during the analysis of soil samples from Mount Tsukuba in Japan as a fermentation product of the fungus Streptomyces tsukubaensis. Oral bioavailability is variable ranging from 5 % to 67 % with the highest rate of absorption under fasting conditions and the lowest rate under high-fat meal intake. Unlike CsA, Tac absorption is not influenced by the presence of bile, which may be beneficial in liver transplant recipients. Tac was first sold as Prograf® and later in a different formulation allowing administration once daily as Advagraf® by Astellas.

Cell cycle inhibitors interfere with clonal expansion of T cells following solid organ transplantation. These include the antimetabolites of purine synthesis, mycophenolate acid (MPA), and inhibitors of *mammalian target of rapamycin* (mTOR).

Antimetabolites of purine were used from the beginning of solid organ transplantation. Calne introduced *6-mercaptopurine* in solid organ transplantation, which was soon replaced by the less toxic imidazolyl derivate *azathioprine* (*Aza*). Aza used to be one of the cornerstones of immunosuppression in liver transplantation, but due to its severe side effects, in particular myelosuppression and hepatotoxicity, Aza has now been almost completely replaced by mycophenolate acid. Aza inhibits cell proliferation by interfering with DNA synthesis and has been described to block downstream effects of *cluster differentiation 28* (CD28) by modulating the G protein *ras-related C3 botulinum toxin substrate 1* (Rac1) activity, converting costimulatory into apoptotic signals.

Mycophenolate acid (*MPA*) was first discovered in 1893 by Gosio as a morpholinoethyl ester of mycophenolate produced by the fungus Penicillium. In 1969, MPA was shown to inhibit *type 2 inosine-monophosphate dehydrogenase* (IMPDH), an essential enzyme for lymphocytes to synthesize guanosine nucleotides necessary for DNA replication and proliferation of T and B cells, which are, unlike other cell types, unable to utilize an alternate pathway. MPA inhibits the cell cycle progression from the S to the G2 phase, antibody formation, adhesion molecules, and smooth muscle cell proliferation.

Mycophenolate mofetil (*MMF*) (*CellCept®*, *Hoffmann-La Roche*) is rapidly absorbed in the stomach and converted in the liver to MPA, whereas *EC-MPS* (*Myfortic®*, *Novartis*), a delayed release form of MPA, is released in the small intestine via a pH-dependent dissolution. MPA is conjugated in the liver to the inactive metabolite MPAG, which is excreted primarily in the urine (around 90 %). Therefore, liver disease may impair MPA conjugation and prolong the half-life time of MPA. Dose reduction or even withdrawal due to dose-related gastroenteral side effects or myelosuppression is high, ranging from 24 % to 57 %.

mTOR inhibitors used in solid organ transplantation include *sirolimus* (Rapamune®, Pfizer) and its derivate *everolimus* (US: Zortress®, EU: Certican®, Novartis), which has a shorter half-life time. Sirolimus was discovered in 1975 in the soil of the Easter Island, also called Rapa Nui, giving sirolimus its other name, rapamycin, as a product of the fungus Streptomyces hygroscopicus.

mTOR inhibitors act as antiproliferative agents by binding to FKB12, the same protein that Tac binds to. In contrast to Tac, mTOR inhibitors do not inhibit calcineurin, and both drugs act synergistically. The complex formed by sirolimus and FKBP12 inhibits mTORC1 (*mammalian target of rapamycin complex 1*) resulting in cell cycle arrest in the G1 to S phase

transition, an effect also observed in tumors. mTORC1 is also essential for the development of conventional effector T cells, and sirolimus has been shown to prevent B-cell differentiation into plasma cells with subsequent reduction in the production of antibodies (IgM, IgG, and IgA). In addition to their immunosuppressive functions, mTOR inhibitors show antifibrotic effects by inhibition of hepatic stellate cell proliferation. Sirolimus also blocks *phosphoinositide-3-kinase* (PI3K)*/protein kinase B* (PKB, syn: AKT)-dependent tumor growth and displays antiangiogenic properties by inhibition of vascular endothelial growth factor (Guba et al. 2002). In contrast to CNI, mTOR inhibitors are thought to be not nephrotoxic. However, this has recently been questioned in patients with renal impairment, and mTOR inhibitors were shown to potentiate CNI-induced nephrotoxicity if used in combination. As mTOR inhibitors affect wound healing, they are usually introduced 4–6 weeks after transplantation. Other side effects include elevated blood lipids, leg edema, and mouth ulcers, while initial reports of increased hepatic artery thrombosis could not be confirmed in later studies (Dunkelberg et al. 2003). Although approved for renal and heart transplantation, both sirolimus and everolimus are not approved as monotherapy for liver transplantation. However, several studies are currently testing both compounds, in particular in patients with hepatocellular carcinoma or preexisting renal dysfunction.

Antibodies used in liver transplantation are heterogeneous in their origin, target, and mode of action. They can be subdivided in polyclonal antibodies isolated from animals previously challenged with certain antigens and monoclonal antibodies. Currently, around 20 % of liver recipients receive either polyclonal or monoclonal antibodies. The advantage of monoclonal antibodies is their specificity, selectivity, safety, and lack of toxicity in comparison to other drugs such as CNI or mTOR inhibitors.

Polyclonal antibodies include *antilymphocyte serum* (ALS), *antilymphocyte globulin* (ALG), *equine antithymocyte globulin* (eATG) (Atgam®, Pfizer), and *rabbit antithymoglobulin* (rATG) (Thymoglobulin®, Genzyme). ALS and ALG were successfully used at the beginning of liver transplantation. The mechanisms of action of polyclonal antibody sera and globulins are not fully understood, but it is thought that they cause the clearance of T cells from the circulation and modulate T-cell activation, homing and cytotoxic activities. rATG induces a more intense immunosuppression than eATG with less infusional side effects (fever, hypotension, serum sickness) (Brennan et al. 1999). While ALS and its globulin fraction ALG were produced by priming a species with human splenocytes, thymuses, or lymph node cells, antithymocyte globulins are produced by priming a species with human thymocytes/lymphocytes resulting in a more specific effect against T cells with less side effects caused by other proteins/antibodies.

eATG and rATG are approved for the treatment of acute rejection in renal transplant recipients but are also used to prevent and treat acute rejection of liver and other organ transplants. Side effects include fever, chills, leukopenia, pain, headache, diarrhea, hypertension, nausea, thrombocytopenia, peripheral edema, infection, dyspnea, and tachycardia in up to 25 % of patients. In addition, an increased risk of infections and the development of posttransplant lymphomas have been associated with the application of polyclonal antibodies.

Monoclonal antibodies target different antigens and display different kinds of action. *Muromonab* (OKT3®, Janssen-Cilag) is a murine-depleting anti-CD3 antibody-targeting T cell. Side effects mirror those observed by the cytokine release syndrome and include pulmonary edema, fever, rigor, and gastrointestinal toxicity. *Basiliximab* (Simulect®, Novartis) and *daclizumab* (Zenapax®, Hoffman-La Roche, discontinued) bind and block the alpha-chain of the IL-2 receptor (CD25) expressed on recently activated T cells and on T regulatory cells. While basiliximab is a chimeric molecule with the variable region of murine and the constant region of human origin, daclizumab is almost completely humanized. Significant reduction in rejection rates without an increase in infectious complications or malignancies was observed in

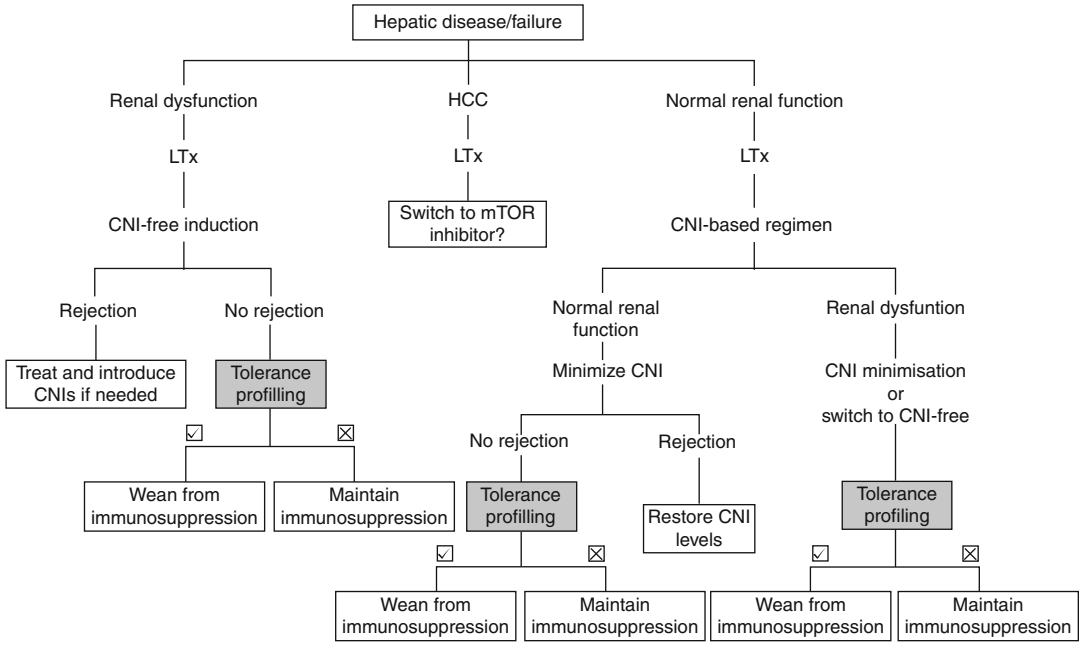
liver transplant recipients in combination with CsA, Aza, and steroids. As these antibodies are highly specific and not depleting, they have only minor side effects. Both are approved for the prophylaxis of acute rejection in renal transplant recipients. *Alemtuzumab* (Campath®, Genzyme) was introduced in 1984 by Waldmann and colleagues. It is a humanized depleting antibody against CD52 expressed on mature lymphocytes as well as monocytes and certain DC subsets, while memory lymphocytes and plasma cells seem to be spared. It shows pronounced depletion of lymphocytes in blood and lymph nodes. The most important side effects are infections due to the severe leukopenia.

Immunosuppression in Liver Transplantation

Despite the existence of very potent agents, immunosuppressive treatment remains a challenge as the use and combination of immunosuppressive drugs has to be adjusted to the patients' underlying disease and conditions like renal impairment or history of hepatocellular carcinoma (see Fig. 2, adopted from Geissler and Schlitt (2009)). It is generally accepted that a higher dose of immunosuppression needs to be given in the early phase after transplantation (*induction phase*) and often a combination of steroids, a CNI and an antiproliferative agents, (MPA or Aza) is given. In addition, an antibody (e.g., basiliximab) can be administered with the aim of lowering the dose of immunosuppressive drugs. In the *maintenance phase*, a cornerstone immunosuppressant (CNI or mTOR inhibitor) with or without low-dose steroids plus antiproliferative agent (MPA/Aza) is often used. *Acute rejection* episodes, suspected or histologically proven, need more intensive treatment, which is typically performed by the administration of high-dose steroids, e.g., methylprednisolone 500–1,000 mg over 3 days. If rejection persists (so-called steroid-refractory rejection), polyclonal (eATG, rATG) or depleting monoclonal (muromonab, alemtuzumab) antibodies are given. If rejection is suspected to be mediated by host antibodies, plasmapheresis and/or the administration of rituximab (MabThera®, Hoffman-La Roche; Rituxan®, Biogen Idec/Genentech), an anti-CD20 antibody, may be considered.

Typical immunosuppressive protocols use a combination of two or three different drugs with or without an antibody in the induction phase. Despite nephrotoxicity and other severe side effects, CNI remain the cornerstone in the treatment of liver transplant recipients. A meta-analysis could demonstrate an advantage of Tac over CsA with regard to overall survival (Haddad et al. 2006), and therefore, most liver transplant centers use Tac. In addition to CNI, a cell cycle inhibitor is often added. Due to its better side effect profile, MPA is preferentially used. High doses of methylprednisolone (500–1,000 mg/d) are typically given just prior to reperfusion followed by rapid tapering. Steroids can be continued on a low dose (typically 25–50 mg or less/d), but many centers try to completely wean steroids to avoid long-term side effects like hypertension, diabetes mellitus, osteoporoses, and cataract. According to the Scientific Registry of Transplant Recipients (SRTR, www.srtr.org), the most frequently used maintenance therapies in the United States were Tac in around 90 %, steroids round 85 %, and MMF in around 60 % of liver transplant recipients with stable immunosuppression for 6 months (Toso et al. 2010). Although mTOR inhibitors are potent immunosuppressive agents in liver transplantation, a warning by the FDA was announced in November 2009: a trial by the manufacturer (*NCT00086346) showed increased mortality in stable liver transplant patients after conversion from CNI to sirolimus. Therefore, mTOR inhibitors should currently be restricted to clinical trials, patients that are not suitable for CNI (e.g., due to renal impairment), and patients with cancer (e.g., hepatocellular carcinoma) due to its antiangiogenic and antiproliferative properties.

Renal impairment is frequently observed in patients with hepatic failure and decompensated cirrhosis (hepatorenal syndrome). In addition, organ allocation programs based on the Mayo End-Stage Liver Disease (MELD) score system,



Immunosuppression in Clinical Liver Transplantation, Fig. 2 Suggested algorithm for immunosuppressive treatment in liver transplant recipients (Adopted from Geissler and Schlitt 2009)

which includes renal function (serum creatinine) as one of the parameters to assess the urgency of liver transplantation, favor patients with impaired renal function. Also, early acute renal dysfunction is frequent after liver transplantation and associated with high morbidity and mortality. The use of antibodies (eATG/rATG or basiliximab) during the induction phase has demonstrated reduced requirement of CNI over time and therefore minimizes the risk for CNI-induced side effects like renal impairment. However, although antibodies effectively induce immunosuppression, complete cessation of CNI is associated with an increased risk of acute rejection. Another approach is to keep patients on MPA and low-dose CNI. MPA monotherapy with complete cessation of CNI is possible but has been associated with an increased risk of acute rejection (Schlitt et al. 2001; Stewart et al. 2001). Until recently, mTOR inhibitors were thought to not be nephrotoxic and here was a hope that they could reverse renal dysfunction. Although an initial study supported this view, later studies showed only minor improvement. Therefore, mTOR inhibitors should be introduced rather early after wound healing has been completed (typically 4–6 weeks after transplantation) before irreversible renal damage has occurred.

Hepatitis C virus (*HCV*) *infection*-induced liver cirrhosis has become the leading cause for liver transplantation in many countries, and reinfection of the new graft frequently occurs. After transplantation, the outcome is heterogeneous. Immunosuppression, as seen in HIV co-infected patients, seems to be associated with progressive disease requiring a new graft within 5–7 years after transplantation. The effects of immunosuppressive agents on the recurrence and progression of HCV-induced cirrhosis after transplantation are not fully understood. However, steroids, in particular pulse therapy, are known to accelerate liver damage and fibrosis due to HCV reinfection. A meta-analysis of protocols that taper or completely avoid using steroids in liver transplant recipients showed a significant reduction in HCV recurrence in the steroid-free groups without an increase in acute rejection, when steroids were replaced by MPA or antibodies

(eATG/rATG, basiliximab) (Segev et al. 2008). However, in cases of acute rejection, steroids can safely be used without increasing fibrosis of the graft, when steroid tapering is performed gradually (Vivarelli et al. 2007). When using a steroid-free protocol, it needs to be considered that critically ill liver patients often suffer from hepatoadrenal syndrome/insufficiency, which appears to be associated with a high mortality when no steroids are given.

Some experimental studies suggest that CsA, but not Tac, may inhibit HCV replication, but this could not be confirmed in a clinical meta-analysis (Berenguer et al. 2007). Aza and MPA were compared to their ability to inhibit virus replication using an in vitro system of bovine viral diarrhea virus, a close relative of hepatitis C virus. Aza showed a tenfold higher decrease in replication compared to MPA (Stangl et al. 2004), but, again, clinical trials comparing Aza and MMF treatment in liver transplant recipients could not demonstrate a difference in viral RNA titers (Zekry et al. 2004). Alemtuzumab spares memory lymphocytes and plasma cells, and it was hoped that it may be beneficial in patients with HCV infection. However, the opposite seemed to be the case as an increase in HCV viral load was observed following lymphocyte depletion with alemtuzumab (Marcos et al. 2004). In summary, in HCV-positive patients, the optimal protocol would be one minimizing the use of steroids and consisting of a CNI and either AZA, MPA, or anti-CD25 antibody.

Hepatocellular carcinoma (HCC) accounts for up to 10 % of all liver transplants. Tumor recurrence after transplantation remains a major problem as immunosuppression is thought to inhibit tumor surveillance by the immune system. Except for mTOR inhibitors, all other immunosuppressive drugs have either no (e.g., MPA) or a negative effect on tumor progression (Schlitt et al. 2011). CsA enhanced tumor growth in a TGF-β-dependent mechanism in a mouse model, which was independent off the immunosuppressive function (Hojo et al. 1999). In addition, CNI seem to increase tumor angiogenesis via stimulation of VEGF and is thought to inhibit DNA repair mechanisms. Clinically, both CsA and Tac are associated with high rates of HCC recurrence (Vivarelli et al. 2008).

Sirolimus has been shown to be associated with a beneficial outcome in patients transplanted for HCC (Toso et al. 2010), although expression of phosphorylated mTOR was found in only 15–40 % of HCCs. mTOR inhibitors also possess anticancer effects as they inhibit cell cycle progress and by blocking many growth factors including VEGF which is important in angiogenesis. So far, only retrospective studies analyzed the effect of mTOR inhibitors in patients transplanted for HCC suggesting a beneficial effect. The results of a prospective, randomized, international multicenter study (SiLVER05, *NCT00355862) addressing this question are expected for 2013.

Autoimmune liver diseases can reoccur after liver transplantation, but no clear association between the selection of immunosuppressive agents and the recurrence of autoimmune hepatitis (recurrence rate 22 %), primary biliary cirrhosis (recurrence rate 18 %), or primary sclerosing cholangitis (recurrence rate 11 %) has been found (Gautam et al. 2006). While most centers try to wean steroids within the first year to avoid steroid-induced side effects, it is believed that steroids might prevent or delay the reoccurrence of underlying disease and treatment is therefore often continued. If autoimmune hepatitis affects the new graft, mTOR inhibitors may be beneficial due to their antifibrotic properties, but no study testing this hypothesis has been performed yet.

Trends and Future Outlooks

New immunosuppressive agents are currently tested in solid organ transplantation: several *antibodies* are under investigation in liver transplantation, e.g., *Belatacept* (Nulojix®, Bristol-Myers Squibb) (*NCT00555321), which has shown promising data in kidney transplant recipients. Belatacept is a fusion protein comprised of the extracellular CTLA-4 domain and the Fc portion of IgG1, which binds to CD28 expressed on

T cells and therefore blocks co-stimulation by CD80 and CD86. Another promising antibody was *efalizumab* (Raptiva®, Merck Serono), a nondepleting monoclonal humanized antibody against LFA-1 expressed by both T and B cells. The blockage of LFA-1 inhibits the formation of the immunological synapse of lymphocytes to the antigen-presenting cell, and efalizumab did not only inhibit T-cell activation but also prevented alloantibody responses. Efalizumab was initially approved for psoriasis and tested in kidney transplant recipients but was withdrawn from the market in 2009 due to increased risk for the development of progressive multifocal leukoencephalopathy. *Small molecules* interfering with intracellular signaling have become of major interest in the transplant field. Although treatment with *fingolimod* (FTY720, Gilenya®, Novartis), an antagonist for sphingosine-1 phosphate signaling important for lymphocyte homing, had to be stopped in transplantation trials due to severe side effects, it illustrated the fact that new drugs targeting novel pathways are arising. Other promising molecules include *tofacitinib* (CP-690550, no assigned trade name, Pfizer) that targets the Janus tyrosine kinase 3, which associates with the common gamma chain and therefore may inhibit IL-receptor signaling, including IL-2 signaling, and *sotrastaurin* (AEB071, no assigned trade name, Novartis) (*NCT01128335) that targets protein kinase C, which is important in T-cell activation and IL-2 production.

Cell-based therapies in solid organ transplantation are becoming increasingly popular due to encouraging results that were obtained in graft-versus-host disease following allogeneic stem cell transplantation. Their advantage is that cells are able to migrate to certain anatomical sites and interact with the immune system in an antigen-specific way. It is hoped that this approach might help to minimize the necessity of currently used agents. However, cell-based therapies are still in the experimental setting and neither the cell type(s) used nor the number of cells that needs to be administered nor the route of administration are clearly defined. Examples of cell types currently tested are *T regulator cells* (Tregs), *mesenchymal stem cells* (MSC), *multipotent adult progenitor cells* (MAPC), *regulatory macrophages* (Mregs), dendritic, and other cells. An international study (ONE Study, www.onestudy.org) is currently trying to define the most potent cell type in solid organ transplantation using kidney transplant recipients. However, a major problem in the use of cells is that their production is often not standardized, and therefore, the comparison of reports from different centers is almost impossible. In addition, the application of living cells, which have been expanded ex vivo, may carry the risk of malignant transformation, in particular as not the cells with the highest immunosuppressive potency but cells with the highest proliferation rate preferentially expand.

Induction of immune tolerance, which has been observed to occur spontaneously after liver transplantation in a variety of species including pigs, rats, and mice, is still the holy grail of solid organ transplantation. However, despite the discovery of several tolerogenic properties of liver allografts (Benseler et al. 2007) and reports of humans with stable liver function completely off immunosuppression, antigen-specific tolerance cannot be induced in the human setting in a reliable and predictable way. However, some evidence exists, which agents are more likely to promote or inhibit the induction of tolerance. Small animal studies demonstrated that T-cell activation is an important step in the active process of antigen-specific tolerance induction, and agents that inhibit T-cell activation like CNI are thought to inhibit rather than promote tolerance. Contrary to CNI, mTOR inhibitors are thought to promote tolerance, as they do not interfere with T-cell activation but prevent proliferation of conventional effector T cells, and promote the induction of Tregs. Treatment with MPA in conjunction with MSC was able to induce tolerance in a small animal model of heart transplantation, but information about liver allografts is missing. The induction of tolerogenic Tregs was reported by the infusion of eATG and rATG, while anti-CD25 antibodies might inhibit tolerance as CD25 is also expressed on Tregs. As alemtuzumab was highly effective in depleting lymphocytes,

it was hoped that alemtuzumab may also induce tolerance. However, the use of alemtuzumab in renal transplant recipients did not lead to tolerance. This might be due to the fact that this drug does not deplete memory lymphocytes.

Conclusion

Immunosuppression in liver transplant recipients is characterized by an individual approach based on the specific side effects of current drugs and the patients' conditions and diseases. Due to their potency, calcineurin inhibitors remain the cornerstone of immunosuppressive therapy in the maintenance phase, but due to their side effects, in particular nephrotoxicity, other agents, e.g., mTOR inhibitors, are currently tested. In addition, the use of monoclonal antibodies during the induction phase may lower the need of subsequent immunosuppressive treatment and thereby decreasing both side effects of drugs and susceptibility of infection. This might be even more realized with the use of molecular agents and cell therapies, a strategy, which might finally lead to the ultimate goal of solid organ transplantation: the induction of immunological tolerance for the transplanted organ.

* www.clinicaltrails.gov

Cross-References

- Acute and Chronic Hepatitis B Virus Infection, Immune Response
- Autoimmune Hepatitis: Pathogenesis, Association with Other Syndromes
- Immune Responses to the Hepatitis C Virus
- Kupffer Cells in Immune Tolerance
- Liver Transplantation for Chronic Viral Hepatitis
- Liver Transplantation Tolerance in Animal Models for Encyclopedia of Medical Immunology
- Mammalian Target of Rapamycin (mTOR)
- NF-κB
- Nuclear Factor of Activated T Cells (NFAT)
- TGF-β
- Transplantation for Autoimmune Liver Diseases
- Tregs in the Liver

References

Benseler V, McCaughan GW, Schlitt HJ, Bishop GA, Bowen DG, Bertolino P. The liver: a special case in transplantation tolerance. Semin Liver Dis. 2007;27:194–213.

Berenguer M, Royuela A, Zamora J. Immunosuppression with calcineurin inhibitors with respect to the outcome of HCV recurrence after liver transplantation: results of a meta-analysis. Liver Transpl. 2007;13:21–9.

Brennan DC, Flavin K, Lowell JA, Howard TK, Shenoy S, Burgess S, Dolan S, Kano JM, Mahon M, Schnitzler MA, Woodward R, Irish W, Singer GG. A randomized, double-blinded comparison of Thymoglobulin versus Atgam for induction immunosuppressive therapy in adult renal transplant recipients. Transplantation. 1999;67:1011–8.

Dunkelberg JC, Trotter JF, Wachs M, Bak T, Kugelmas M, Steinberg T, Everson GT, Kam I. Sirolimus as primary immunosuppression in liver transplantation is not associated with hepatic artery or wound complications. Liver Transpl. 2003;9:463–8.

Gautam M, Cheruvattath R, Balan V. Recurrence of autoimmune liver disease after liver transplantation: a systematic review. Liver Transpl. 2006;12:1813–24.

Geissler EK, Schlitt HJ. Immunosuppression for liver transplantation. Gut. 2009;58:452–63.

Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med. 2002;8:128–35.

Haddad EM, McAlister VC, Renouf E, Malthaner R, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus for liver transplanted patients. Cochrane Database Syst Rev. 2006;4:CD005161.

Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, Shimbo T, Suthanthiran M. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature. 1999;397:530–4.

Marcos A, Eghtesad B, Fung JJ, Fontes P, Patel K, Devera M, Marsh W, Gayowski T, Demetris AJ, Gray EA, Flynn B, Zeevi A, Murase N, Starzl TE. Use of alemtuzumab and tacrolimus monotherapy for cadaveric liver transplantation: with particular reference to hepatitis C virus. Transplantation. 2004;78:966–71.

Post DJ, Douglas DD, Mulligan DC. Immunosuppression in liver transplantation. Liver Transpl. 2005;11:1307–14.

Schlitt HJ, Barkmann A, Boker KH, Schmidt HH, Emmanouilidis N, Rosenau J, Bahr MJ, Tusch G, Manns MP, Nashan B, Klempnauer J. Replacement of

calcineurin inhibitors with mycophenolate mofetil in liver-transplant patients with renal dysfunction: a randomised controlled study. Lancet. 2001;357:587–91.
Schlitt HJ, Mornex F, Shaked A, Trotter JF. Immunosuppression and hepatocellular carcinoma. Liver Transpl. 2011;17(Suppl 2):S159–61.
Segev DL, Sozio SM, Shin EJ, Nazarian SM, Nathan H, Thuluvath PJ, Montgomery RA, Cameron AM, Maley WR. Steroid avoidance in liver transplantation: meta-analysis and meta-regression of randomized trials. Liver Transpl. 2008;14:512–25.
Stangl JR, Carroll KL, Illichmann M, Striker R. Effect of antimetabolite immunosuppressants on Flaviviridae, including hepatitis C virus. Transplantation. 2004;77:562–7.
Stewart SF, Hudson M, Talbot D, Manas D, Day CP. Mycophenolate mofetil monotherapy in liver transplantation. Lancet. 2001;357:609–10.
Toso C, Merani S, Bigam DL, Shapiro AM, Kneteman NM. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. Hepatology. 2010;51:1237–43.
Vivarelli M, Burra P, La Barba G, Canova D, Senzolo M, Cucchetti A, D'Errico A, Guido M, Merenda R, Neri D, Zanello M, Giannini FM, Grazi GL, Cillo U, Pinna AD. Influence of steroids on HCV recurrence after liver transplantation: a prospective study. J Hepatol. 2007;47:793–8.
Vivarelli M, Cucchetti A, La Barba G, Ravaioli M, Del Gaudio M, Lauro A, Grazi GL, Pinna AD. Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. Ann Surg. 2008;248:857–62.
Zekry A, Gleeson M, Guney S, McCaughan GW. A prospective cross-over study comparing the effect of mycophenolate versus azathioprine on allograft function and viral load in liver transplant recipients with recurrent chronic HCV infection. Liver Transpl. 2004;10:52–7.

Impact of Recurrent Autoimmune Diseases in Renal Transplant Outcomes

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Synonyms

Diseases affecting transplant renal allografts; Relapsing glomerulonephritis

Definition

Glomerulonephritis encompasses various autoimmune diseases, either systemic or renal limited, that affect renal function and may lead to end-stage renal disease. These diseases may relapse in the transplanted kidneys causing dysfunction or even loss of function.

Introduction

Primary and secondary glomerular diseases are some of the leading causes of advanced chronic kidney disease (CKD) worldwide leading to renal replacement therapy. As with any other etiology of end-stage renal disease (ESRD), kidney transplantation is the preferred choice of renal replacement therapy (RRT) in patients with advanced CKD caused by glomerulonephritis (GN). Transplantation improves the longevity as well as the quality of life compared to dialysis in these patients.

The paradigm of posttransplant recurrence of GN is highly intriguing for at least two reasons. The concept of placing an allograft into immunologically hostile environment is in itself challenging, and to consider adding the risk of a recurrent GN because of the ostensibly persistent pathogenetic mechanisms is even more so. Secondly, counterintuitive to the original notion, these immunological diseases relapse in the allograft in the face of maintenance immunosuppression which is sufficiently intensive to prevent acute rejection. This is probably an illustration of the complex pathogenetic pathways and redundancy of the immune system.

Due to the immunological mechanisms of the GN, disease recurrence in the transplant kidney is well recognized and potentially affects the allograft function. Risk factors for recurrence must be identified during the pre-transplant evaluation based the specific disease and discussed with the patient. All attempts must be made to explore strategies to minimize the risk of recurrence and its impact on the allograft function. With the availability of more potent immunosuppression therapies in the past two decades, acute rejection has become an uncommon cause for graft loss,

and therefore, the attention is now being directed towards strategies to improve long-term allograft function. As will be seen in the later sections, recurrent GN represents an important cause of allograft loss, and it is critical to have in place surveillance protocols to prevent, identify, and treat recurrent disease.

Many systemic diseases, such as diabetes mellitus, hypertension, Fabry's disease, and primary hyperoxalosis, would also obviously affect the renal allograft function either very early or in the long term. However, the discussion in this entry will be limited to immunologically mediated diseases that ultimately lead to various types of GN.

That the underlying GN recurs in the allograft was observed since the days of successful kidney transplantation. The first successful kidney transplant from 1954, performed between identical twins, functioned for more than 9 years without requiring immunosuppression before failing from recurrent "Bright's disease," the eponym used to describe all glomerulonephritis before specific GN were described with the advent of techniques such as immunofluorescence and electron microscopy. Glassock et.al in 1968 published the first classic description of recurrent GN in kidney transplant patients using microscopic techniques, thus raising awareness of such recurrence posttransplantation (Glassock et al. 1968).

One of the unique but indirect benefits of kidney transplantation in patients with GN is the ability to study the natural history and pathogenesis of the various GN in the setting of a new kidney. This has certainly been the case with focal segmental glomerulosclerosis (FSGS) with many new insights gained recently with regards to its pathogenesis when studied in the context of transplantation.

Epidemiology

The true prevalence of GN in pre-transplant patients is difficult to estimate because of the underutilization of diagnostic kidney biopsies. This, in turn, is because either the etiology is presumed given the appropriate clinical context (e.g., diabetes mellitus) or clinical presentation is very late with low diagnostic yield from a biopsy. Even among posttransplant recipients, a recurrent GN is only suspected in case of renal dysfunction and then confirmed by a biopsy. Patients with stable renal allograft function but with subclinical recurrent GN may not come to the clinician's attention.

Despite this conundrum, recurrent GN is now well known to be an important cause of death-censored graft loss in the long term. In a study of 1,505 cases with both native kidney and kidney allograft biopsies documenting recurrent glomerular disease, graft loss due to recurrent GN was the third most frequent cause for graft failure 10 years after kidney transplantation (Briganti et al. 2002). The clinical presentation and course are dependent on the individual disease and highly variable between diseases. In the majority of the cases, this occurs as a subacute or chronic event with the only exception being the FSGS, in which recurrence can be "catastrophic," that is, immediately following transplantation sometimes as early as within 1–2 days. In the absence of aggressive and immediate treatment, this type of recurrence can potentially lead to "primary nonfunction" and ultimate graft loss.

Clinical Presentation

While GN encompasses a wide variety of individual diseases, certain general remarks can be made with regard to the pattern of recurrence posttransplantation, with the exception of FSGS which will be discussed in more detail later. In others, the recurrence is subacute over several months to a few years posttransplant. In some instances, proteinuria is the sole manifestation of disease recurrence. Subclinical cases without appreciable proteinuria are discovered fortuitously when kidney biopsies are done for other indications. There may not be any appreciable changes in serum creatinine initially, but it is now well accepted that recurrent GN leads to earlier allograft loss than in those without recurrence. Very occasionally, recurrence may present as a rapidly progressive glomerulonephritis (RPGN) which may then lead to early graft loss.

Screening and Diagnosis

Routine monitoring for recurrent GN is recommended in all renal transplant patients regardless of the native kidney etiology for the following reasons:

1. Native kidney biopsy is not available in all the patients either because of their late presentation with advanced CKD or because the diagnosis is presumed (e.g., diabetes mellitus, autosomal dominant polycystic kidney disease). These individuals may have had GN as the primary disease or as a coincidental comorbid condition.
2. De novo glomerulonephritis may occur posttransplant. Membranous GN and IgA nephropathy are well known to occur de novo posttransplant.

Screening is valuable because recurrent disease may affect allograft function, and with early detection and treatment, it is possible to delay or prevent disease progression. With increasing experience in kidney transplantation, with evolving practices and treatment protocols, it has become clear that disease recurrence in allografts is very difficult to predict and that clinical presentation can be highly variable. Since GN recurrence is clinically silent in the majority of cases, screening is recommended on a timely basis to detect, confirm, and treat these recurrences.

1. Detection of proteinuria is perhaps the only reliable, inexpensive, and noninvasive method to monitor and detect disease recurrence in transplant kidneys. This monitoring is done with urine dipstick followed by urine protein/creatinine ratio or timed urine collection. Since FSGS is known to recur early and aggressively, it is recommended that screening be done frequently – daily for the first 1 week, weekly for 4 weeks, every 3 months for 1 year, and then annually. For all the other diseases, a less frequent monitoring is recommended – once during the first month, every 3 months during the first year, and then annually (KDIGO 2009).
2. Timed protocol biopsies are practiced in some centers as a tool to detect subclinical acute rejection, at least for the first few years. A coincidental detection of disease recurrence may also be an added benefit with such a practice.
3. Once proteinuria is detected, with or without graft dysfunction, a renal biopsy is recommended. In addition to the routine H&E staining, immunofluorescence and electron microscopy may be valuable depending on the underlying GN.
4. Once a disease is identified, supporting evidence may be obtained by various serological studies (e.g., anti-glomerular basement membrane (GBM) antibodies or antineutrophil cytoplasmic antibodies (ANCA) may be ordered).

Treatment

In general, treatment of recurrent diseases is unsatisfactory as is the case with GN in general. Some of the available treatments are discussed under the individual GN with controversies highlighted. Regardless of the etiology of posttransplant GN, anti-proteinuric therapies are generally recommended and usually effective in limiting the amount of proteinuria. These therapies include drugs like ACE inhibitors and angiotensin receptor blockers, optimal control of hypertension, low protein diet, and other less proven therapies such as maintenance of hemoglobin and lipid levels within normal range.

For the sake of convenience, the specific description of each GN will be discussed under the broad categories of primary and secondary glomerular disease. Also, to stay within the scope of this entry, discussion will be centered on the patterns of recurrence and pathogenic mechanisms. Discussion will also be limited to the recent advances in this field and concepts that have wide acceptance.

Primary (Idiopathic) Glomerular Diseases

Focal Segmental Glomerulonephritis

Idiopathic FSGS tends to recur in about 30 % of all first renal allograft recipients (Couser 2005).

The rate approaches 100 % with subsequent allografts if the first allograft was lost due to recurrent disease. Recurrence is unpredictable with any individual case, and currently there are no treatments that are known to prevent recurrence, including preemptive plasmapheresis. Interestingly, recurrence is uncommon in familial forms of FSGS indicating different pathogenic mechanisms at play in the causality. Clinically, two patterns of recurrence are well recognized: (a) a catastrophic early recurrence with massive proteinuria that leads to anuria and graft loss in the absence of aggressive treatment and (b) a subacute recurrence characterized by gradual increase in proteinuria over months to years. The relative risk of allograft loss with recurrent disease is as high as 2.25 times compared to those without (Hariharan et al. 1999).

A circulating factor in the serum causing disease recurrence was postulated long ago and further strengthened by recent experimental data. The reasons behind this hypothesis are several: (1) relapse of the disease in transplant recipients is rapid and frequent, (2) aggressive plasma exchange or immunoadsorption using staphylococcal A columns lead to remission in many cases indicating that the pathogenic factor is amenable for removal, (3) injection of the FSGS plasma removed by apheresis into experimental animals can cause proteinuria immediately (Sharma et al. 2002), and (4) use of anti-B cell therapies led to disease remission in some cases. However, despite years of intense research, the nature of putative circulating factor is only recently being unraveled. A circulating soluble urokinase receptor (suPAR) is now shown to be not only elevated in subjects with FSGS before transplant but is also associated with higher disease recurrence following transplantation (Wei et al. 2011). These investigators demonstrated, using mouse models, that circulating suPAR activates podocyte β_3 integrin in both native and grafted kidneys, causing foot process effacement, proteinuria, and FSGS-like glomerulopathy. Renal disease developed only when suPAR sufficiently activated podocyte β_3 integrin leading to the hypothesis that the disease can be abrogated by lowering serum suPAR concentrations.

Patients with familial forms of FSGS have mutations of genes encoding for various podocyte slit-diaphragm proteins such as podocin, nephrin, and α-actinin-4. Up to 8 % of these patients may also develop recurrent FSGS although the pathogenic mechanism is not well understood. Disease relapse based on a circulating factor is supported by an experimental rat model. The Buffalo/Mna rat develops FSGS because of a defective autosomal recessive gene. The gene, *Pur1*, is mapped to chromosome 13 in a region syntenic with the long arm of chromosome 1 which contains the gene coding for the podocin, a podocyte protein with a major role in selective filtration function. When affected kidneys were transplanted into normal Lew.w1 rats, proteinuria and renal lesions disappeared (Bertelli et al. 2003). But when normal Lew.w1 kidneys were transplanted into Buffalo/Mna rats, proteinuria occurred, possibly by developing a de novo alloantibody against the normal podocin. This mechanism might explain why some of the familial FSGS cases have disease relapse following transplantation.

Currently available treatments are controversial, unsatisfactory and not consistently effective. These include high-dose intravenous cyclosporine, plasma exchange, and rituximab. In addition to the well-known inhibition of cytokine production by the T cells, cyclosporine was reported to inhibit calcineurin-mediated dephosphorylation of synaptopodin, a protein critical for stabilizing the actin cytoskeleton in podocytes. Corticosteroids have not been shown to be consistently effective in either achieving or maintaining remission. Rituximab was reported to be successful in achieving remission but was ineffective in many other reports. With any of these therapies, only approximately 60–70 % of patients achieve complete or partial remission.

While the mechanism for rituximab's benefit in the treatment of recurrent FSGS is not clear, recent experimental data indicate that it may operate in a B cell-independent fashion. Patients who had recurrent FSGS were found to have fewer podocytes with sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) protein in kidney biopsies. This disrupts the actin cytoskeleton

and leads to loss of podocyte function. Rituximab binds to the SMPDL-3b and prevents its downregulation. This may lead to preserved podocyte viability and prevention of FSGS recurrence (Fornoni et al. 2011).

Immunoglobulin A Nephropathy (IgAN)

IgAN usually has an indolent clinical course and causes ESRD in 30–50 % individuals after many years of disease progression. It is characterized by the deposition of aberrant galactose-deficient IgA_1 in the glomerular mesangium as aggregates or as IgA-IgG immune complexes. Recurrent IgAN is an example of a classical subclinical presentation of a glomerular disease where patients have only microscopic hematuria and proteinuria without significant allograft dysfunction. This may not be an indication for renal biopsy at many centers and therefore the recurrence may be missed. The relapsed disease takes many years to progress, and the longer patients are followed, the higher the chances of discovering or recognizing a recurrence. Posttransplant recurrence probably occurs in about 33 % individuals, but reported recurrences were highly variable, between 9 % and 61 % (Ponticelli et al. 2004). Recurrence tends to occur in younger individuals and those with rapid progression of original disease, but as with other GN, this is hard to predict.

In addition of recurrent disease, there appears to be another pathogenic mechanism at play in a small number of individuals. Recipients of either deceased or living kidneys with preexisting "hidden" IgA deposits at the time of transplant may develop de novo IgA nephropathy (Suzuki et al. 2009). The putative mechanism is that people with circulating antibodies to the abnormal IgA would react to the "planted" IgA deposits in the donor kidney triggering an IgAN-like disease. Unfortunately, at present there are no commercially available assays to measure the titers of such circulating autoantibodies.

While it is widely accepted that the type and intensity of immunosuppression protocols do not influence the posttransplant recurrence of IgAN, a few single center reports suggest that either steroid avoidance protocols or steroid withdrawal in late posttransplant period may be associated with higher recurrence rate as well as faster disease progression. However, only large randomized controlled trials can lead to any useful conclusions in this matter. In those few instances of rapidly progressive recurrent IgAN intense immunosuppression with high-dose corticosteroids, cyclophosphamide and/or plasmapheresis is suggested but is not of proven benefit.

There are many overlapping pathological and clinical features between IgAN and Henoch-Schönlein purpura (nephritis) HSP, but a few unique features of HSP deserve discussion: (a) it is a systemic small vessel vasculitis involving many extrarenal organs including skin, joints, and the gastrointestinal tract; (b) recurrent disease with crescents and necrotizing lesions have a very rapid and high incidence of graft loss; (c) a circulating IgA-ANCA (antineutrophil cytoplasmic antibody) has been reported in some patients and seems to have a high correlation with early recurrent disease; and (d) recurrence is very high (up to 75 %) in those patients who had a rapid progression of native disease with necrotizing, crescentic lesions on biopsies.

Various reports indicate that histological recurrence is very common, similar to the IgAN. One study reported a 78 % recurrence at 5-year follow-up (Meulders et al. 1994). However, another study with a 15-year follow-up reported similar graft survival between those with or without recurrent HSP and a 42 % incidence of recurrence. Among those with recurrent disease, graft loss was almost 50 % (Moroni et al. 2008). Histological findings included IgA immune complex deposits in the mesangium, focal/segmental necrotizing lesions, and in some cases crescents. Early clinical features include hematuria, moderate proteinuria, and hypertension. Treatment of recurrent disease is unsatisfactory at present.

Idiopathic Membranous Nephropathy (IMN)

IMN is characterized histologically by the subepithelial deposition or accumulation of immune complexes. This leads to the thickening and loss of integrity of the basement membrane. Like IgAN, IMN has a slow, indolent course that leads to ESRD in about 40–50 % of patients. Recurrence of IMN is typically observed 2–3

years posttransplant. But the rate of recurrence is probably much higher than reported because of the widely varying indications for kidney biopsy. Another confounding factor is the development of de novo IMN in allografts, which histologically is almost identical to IMN.

While the mechanisms of recurrent IMN are still unclear, recent advances suggest IMN patients have autoreactivity to podocyte proteins, such as neutral peptidase and aldose reductase (Prunotto et al. 2010). Alloreactivity to podocyte antigens is possible in allografts. This was confirmed when anti-phospholipase A_2 receptor antibodies were found to be responsible for posttransplant IMN recurrence (Stahl et al. 2010).

The clinical outcomes for allografts that experience IMN recurrence are similar to those of nonrecurrence. Overall prognosis, similar to any recurrent GN, is determined by the degree of proteinuria.

Treatment of IMN is basically empiric. No specific therapy was proven to be beneficial including corticosteroids. Rituximab was reported to be beneficial in some patients but this is anecdotal.

Membranoproliferative Glomerulonephritis (MPGN)

MPGN includes a complex combination of diseases and can be either idiopathic or secondary to chronic infections, cryoglobulinemia, or systemic autoimmune disorders. A large variety of other etiologies also result in the clinical phenotype of MPGN. Pathogenesis involves chronic complement activation and depressed complement levels. The end result of these varying etiologies is the deposition of immune complexes in glomeruli, diffuse proliferative lesions, and widening or duplication of the basement membrane. Based on the histological findings, MPGN is divided into three types. Recurrence after transplantation is common in all the types. It is sometimes difficult to distinguish MPGN from a similar appearance in transplant glomerulopathy. Clinical context and serological studies help in separating MPGN from transplant glomerulopathy.

Recurrent MPGN type I is reported in 20–30 % of cases and may present not only with nephrotic syndrome but in some cases rapid decline in renal function. Therefore, it can have significant impact on graft survival. Histopathology demonstrates subendothelial deposition of immune complexes containing C3 and IgG. The mechanism of recurrence is presumably the ongoing systemic disease that caused the original kidney lesions, such as hepatitis C infection or hereditary complement deficiency leading to chronic low-grade complement activation.

MPGN type II, also known as dense deposit disease (DDD), is characterized by the replacement of the glomerular basement membrane by an extremely dense band of homogenous material ("dense deposits") consisting of C3 and other complement products but without immunoglobulins. The major defect is the excessive activation of the alternative complement pathway. Recurrence of MPGN type II is very high with 80–100 % of the patients developing it. This doubles the relative risk of graft loss. A monoclonal antibody to the C5a complement split product is being studied to prevent posttransplant MPGN recurrence and may have a crucial role as part of induction treatment at the time of transplant. Patients with genetic deficiencies of either factor H or I may benefit from having combined liver and kidney transplantation which would then restore the deficient factors.

Since prevention and treatment are difficult at present, pre-transplant investigation to identify active disease and counseling for possible graft loss is very important. Patients with persistently low complement levels are at the highest risk, and transplantation may have to be postponed until all possible efforts are made to treat the underlying disease (e.g., hepatitis C-related cryoglobulinemia, monoclonal gammopathies) first.

Secondary Glomerular Diseases

Lupus Nephritis

Approximately 30 % of all patients with established lupus nephritis (LN) progress to ESRD, most commonly the class IV disease with proliferative lesions. The onset of renal failure in LN seems to be associated with partial or complete

resolution of extrarenal and serological manifestations of systemic lupus erythematosus (SLE) in many but not all the patients. The specific mechanisms for this phenomenon are not known. Recurrence of LN after renal transplant is very rare, reported to be <5 %. However, some studies reported recurrence rates as high as 30 % and 54 %, based on stringent investigations with serologies and renal biopsies processed for immunofluorescence and electron microscopy. The histologic lesions of recurrent LN are usually mild, mostly consisting of mesangial lesions or atypical pauci-immune proliferative GN, even in studies that employed protocol biopsies. However, histological recurrence is not commensurate with clinical symptoms or signs.

The clinical course of recurrent lupus nephritis is usually indolent and does not seem to have any impact on the allograft function. Likewise, different immunosuppression regimens do not confer any specific advantages, even with steroid avoidance protocols.

Two precautionary strategies are probably critical in peri-transplant management. Patients receiving aggressive immunosuppressive therapies may benefit from delaying transplantation while receiving dialysis, to reduce complications from cardiovascular and infections from prior immunosuppression. Secondly, patients with antiphospholipid antibody syndrome with hypercoagulable syndrome might be at high risk for vascular thrombosis in the peri-transplant period which may include the transplant renal artery leading to early graft loss. Peri-transplant anticoagulation may improve outcomes in such patients.

Amyloidosis

Amyloidosis is a general term describing a group of diseases with deposition of a characteristic proteinaceous material in various organs in the form of fibrils. The principal types that lead to renal involvement are the AL type, with immunoglobulin light chain deposition, and the AA type, with SAA apolipoprotein deposition. Both are systemic disorders affecting many other organs, and therefore, many patients do not get considered for kidney transplantation. AL amyloidosis is particularly a problem because of the high incidence of cardiac involvement.

In the small number of patients with AL amyloidosis who receive transplantation, recurrence has been described frequently, but graft survival appears to be similar to control groups. Patient survival was also comparable in this carefully selected cohort with 95 % 1-year survival and 67 % 5-year survival (Sattianayagam et al. 2010).

AA amyloidosis is caused by many chronic systemic inflammatory diseases, and therefore, recurrence in transplant kidney is based on the specific underlying disease. For instance, presence of rheumatoid arthritis may cause a posttransplant recurrence of AA amyloid deposition in up to 26 %, but there are no reports of recurrence with Behcet's disease.

Combined organ transplantation may be an option for patient with cardiac involvement (heart-kidney) and in those with rare forms of amyloidosis caused by genetic diseases leading to renal involvement (liver-kidney).

ANCA-Positive Vasculitis (Small Vessel Vasculitis)

Small vessel vasculitis (SVV) is another important cause of ESRD and includes several systemic diseases such as Wegener's granulomatosis, Churg-Strauss syndrome, and microscopic polyangiitis. They are all associated with circulating antineutrophil cytoplasmic antibodies (ANCA) which play an important role in the disease pathogenesis. Posttransplant recurrence in kidney in the modern era of immunosuppression is low at 6–7 % cases. Even among the recurrent disease, allograft loss is about 7.7 % at 10 years according to large database trial, which is similar to control population (Briganti et al. 2002). The lower incidence is widely believed to be because of maintenance immunosuppression. Microscopic hematuria and proteinuria are the earliest clinical signs of recurrence. Histopathological findings include focal or diffuse necrotizing glomerulonephritis without immune complexes. Clinical relapse may also occur in extrarenal organs. None of the pre-transplant clinical features including the ANCA tires are useful in predicting recurrence. In fact,

transplantation in spite of persistent high ANCA titers was not detrimental to the allograft. Transplantation should be delayed until clinical remission is maintained for more than a year, since disease relapse within 1 year of treatment is associated with high mortality. A few reports indicate that treatment with cyclophosphamide, plasmapheresis, and rituximab all have a role in achieving successful remission of the recurrent disease but definitive knowledge is lacking.

Anti-glomerular Basement Membrane Antibody Disease (Anti-GBM)

Anti-GBM disease is another interesting clinical paradox in that the native kidney disease is often rapidly progressive and results in ESRD. However, once the disease activity subsides, as evidenced by disappearance of circulating anti-GBM antibodies, transplantation is effective. Recurrence in the allografts is only rarely reported but does occur in the context of increasing anti-GBM antibody titers.

A de novo anti-GBM disease can occur in patients with Alport's syndrome and kidney transplantation. These patients lack the tissue-specific type IV collagen chains in the glomerular basement membrane due to a genetic mutation. These chains, expressed in the normal donor kidney, may elicit an alloimmune response by the recipient. In the presence of conventional immunosuppression, most patients do not develop clinical anti-GBM disease even though histopathologically they can have linear deposition of IgG along the GBM of the transplanted kidney. About 5 % of patients may develop clinical disease indistinguishable from the rapidly progressive Goodpasture's disease but without pulmonary involvement. This probably occurs in the presence of a large gene deletion instead of a point mutation. Evidence suggests that recurrence of anti-GBM disease occurs earlier with subsequent transplants (Browne et al. 2004).

Cross-References

- ▶ Anti-glomerular Basement Membrane Disease
- ▶ IgA Nephropathy
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Transplantation for Autoimmune Liver Diseases
- ▶ Vasculitis: Granulomatosis with Polyangiitis (Wegener's)
- ▶ Vasculitis: Henoch-Schönlein Purpura

References

Bertelli R, Ginevri F, Caridi G, Dagnino M, Sandrini S, Di Duca M, Emma F, Sanna-Cherchi S, Scolari F, Neri TM, Murer L, Massella L, Basile G, Rizzoni G, Perfumo F, Ghiggeri GM. Recurrence of focal segmental glomerulosclerosis after renal transplantation in patients with mutations of podocin. Am J Kidney Dis. 2003;41(6):1314–21. Jun.

Briganti EM, Russ GR, McNeil JJ, Atkins RC, Chadbans J. Risk of renal allograft loss from recurrent glomerulonephritis. N Engl J Med. 2002;347:103–9.

Browne G, Brown PA, Tomson CR, Fleming S, Allen A, Herriot R, Pusey CD, Rees AJ, Turner AN. Retransplantation in Alport post-transplant anti-GBM disease. Kidney Int. 2004;65(2):675–81. Feb.

Couser W. Recurrent glomerulonephritis in the renal allograft: an update of selected areas. Exp Clin Transplant. 2005;3:283–8.

Fornoni A, Sageshima J, Wei C, Merscher-Gomez S, Aguillon-Prada R, Jauregui AN, Li J, Mattiazzi A, Ciancio G, Chen L, Zilleruelo G, Abitbol C, Chandar J, Seeherunvong W, Ricordi C, Ikehata M, Rastaldi MP, Reiser J, Burke GW. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. Sci Transl Med. 2011;3(85):85–ra46. Jun 1.

Glassock RJ, Feldman D, Reynolds ES, Dammin GJ, Merrill JP. Human renal isografts: a clinical and pathologic analysis. Medicine (Baltimore). 1968;47:411–54.

Hariharan S, Adams MB, Brennan DC, Davis CL, First RM, Johnson CP, Ouseph R, Peddi VR, Pelz CJ, Roza AM, Vincenti F. Recurrent and de novo glomerular disease after renal transplantation. A report from the renal allograft disease registry (RADR). Transplantation. 1999;68:635–41.

Kidney Disease Improving Global Outcomes (KDIGO). Care of the kidney transplant recipient. Chapter 10. (2009). [Accessed on 2012 Feb 22] Available from: http://www.kdigo.org/clinical_practice_guidelines/pdf/TxpGL_publVersion.pdf.

Meulders Q, Pirson Y, Cosyns JP, Squifflet JP, van Ypersele de Strihou C. Course of henoch–schonlein nephritis after renal transplantation. Transplantation. 1994;58:1172–86.

Moroni G, Gallelli B, Diana A, Carminati A, Banfi G, Poli F, Montagnino G, Tarantino A, Messa P. Renal

transplantation in adults with Henoch-Schonlein purpura: long-term outcome. Nephrol Dial Transplant. 2008;23:3010–6.
Ponticelli C, Traversi L, Banfi G. Renal transplantation in patients with IgA mesangial glomerulonephritis. Pediatr Transplant. 2004;8(4):334–8. Aug.
Prunotto M, Carnevali ML, Candiano G, Murtas C, Bruschi M, Corradini E, Trivelli A, Magnasco A, Petretto A, Santucci L, Mattei S, Gatti R, Scolari F, Kador P, Allegri L, Ghiggeri GM. Autoimmunity in membranous nephropathy targets aldose reductase and SOD2. J Am Soc Nephrol. 2010;21(3):507–19. Mar.
Sattianayagam PT, Gibbs SDJ, Pinney JH, Wechalekar AD, Lachmann HJ, Whelan CJ, Gilbertson JA, Hawkins PN, Gill-more JD. Solid organ transplantation in AL amyloidosis. Am J Transplant. 2010;10:2124–31.
Sharma M, Sharma R, Reddy SR, McCarthy ET, Savin VJ. Proteinuria after injection of human focal segmental glomerulosclerosis factor. Transplantation. 2002;73(3):366–72. Feb 15.
Stahl R, Hoxha E, Fechner K. PLA2R autoantibodies and recurrent membranous nephropathy after transplantation. N Engl J Med. 2010;363(5):496–8. Jul 29.
Suzuki H, Fan R, Zhang Z, Brown R, Hall S, Julian BA, Chatham WW, Suzuki Y, Wyatt RJ, Moldoveanu Z, Lee JY, Robinson J, Tomana M, Tomino Y, Mestecky J, Novak J. Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. J Clin Invest. 2009;119:1666–77.
Wei C, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, Maiguel D, Karumanchi SA, Yap HK, Saleem M, Zhang Q, Nikolic B, Chaudhuri A, Daftarian P, Salido E, Torres A, Salifu M, Sarwal MM, Schaefer F, Morath C, Schwenger V, Zeier M, Gupta V, Roth D, Rastaldi MP, Burke G, Ruiz P, Reiser J. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nat Med. 2011;17(8):952–60. Jul 31.

Indications for Biopsy in Autoimmune GN

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Synonyms

AKI: Acute kidney injury; ANCA: Antineutrophil cytoplasmic antibodies; Anti-GBM: Anti-glomerular basement membrane; CKD: Chronic kidney disease; GN: Glomerulonephritis; Renal biopsy: Kidney biopsy

Definition

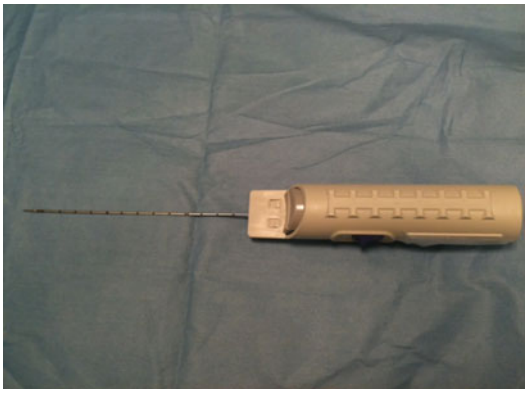
Variety of autoimmune diseases can be associated with renal involvement. Furthermore, these diseases can lead to various clinical presentation including isolated glomerular hematuria, nephrotic syndrome, isolated non-nephrotic proteinuria, and acute nephritic syndrome. A kidney biopsy is an important diagnostic tool and can provide precious information including establishment of the pathological diagnosis, as an aid to decide optimal therapy and to determine the degree of active and chronic changes (Appel 1993).

History

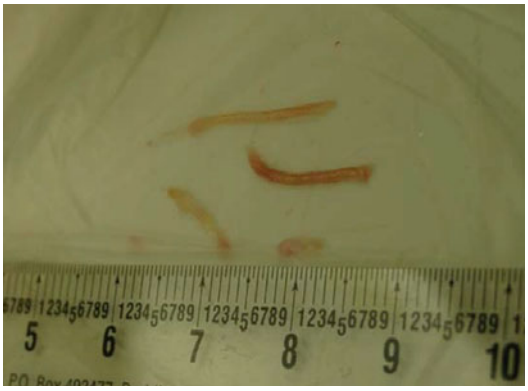
Percutaneous renal biopsy was first described in the early 1950s by Iversen and Brun (reviewed in Iversen and Brun (1997)). Not surprisingly, an adequate tissue diagnosis was achieved in less than 40 % of these early cases given a variety of technical limitations including performing the procedure in the sitting position, use of a suction needle, and intravenous urography for guidance. In 1954, Kark and Muehrcke described a modified technique using a Franklin-modified Vim-Silverman needle with the patient in a prone position (Kark and Muehrcke 1954). An exploring needle was used to localize the kidney before insertion of the biopsy needle. Since then, the basic renal biopsy procedure has remained fundamentally unchanged, although the use of real-time ultrasound and refinement of biopsy needle design have led to considerable improvements both in terms of increasing the diagnostic yield and minimizing procedural complications (Topham and Chen 2010) (Fig. 1).

Diagnostic Utility of the Kidney Biopsy

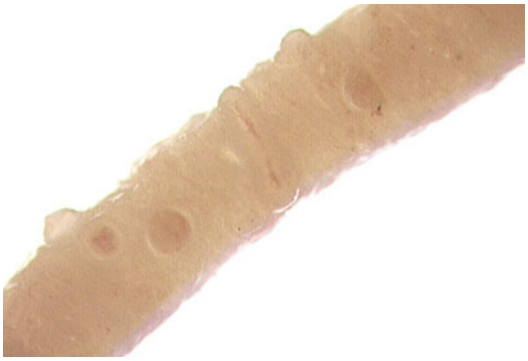
Although renal biopsy is now able to provide a tissue diagnosis in more than 95 % of cases with little risk of life-threatening complications (<0.1 %), very few patients with kidney disease undergo kidney biopsy (Toto 2009). For instance, only a fraction of the patients with a clinical



Indications for Biopsy in Autoimmune GN, Fig. 1 A 22-gauge BARD® disposable core biopsy needle (Source: Ravish Shah)



Indications for Biopsy in Autoimmune GN, Fig. 2 Kidney biopsy core (Source: Gyongyi Nadasdy and Tibor Nadasdy)



Indications for Biopsy in Autoimmune GN, Fig. 3 Kidney biopsy core as seen with a dissecting microscope (note the glomeruli, recognized as round structures) (Source: Gyongyi Nadasdy and Tibor Nadasdy)

diagnosis of diabetic nephropathy undergo a kidney biopsy to confirm the diagnosis (Toto 2009). Yet, when kidney biopsy is performed in patients with type 2 diabetes and kidney disease, more than half reveal a histological diagnosis other than, or in addition to, diabetic nephropathy (Toto 2009). The overall rate of native kidney renal biopsy varies from over 250 procedures per million populations in Australia to less than 75 procedures per million populations in the United States (Briganti et al. 2001). These differences in renal biopsy rate are largely driven by opinion regarding the usefulness of the procedure rather than differences in the spectrum of renal pathology.

Several studies have demonstrated that therapeutic interventions are modified considerably after diagnosis is obtained by a kidney biopsy (Cohen et al. 1989). Renal histopathologic lesions are very difficult to predict based on clinical grounds alone. For instance, in a prospective single center study of 276 native renal biopsies, changes in the management as a direct result of the biopsy occurred in 86 % of cases with nephrotic-range proteinuria, 71 % of cases with acute renal failure, and 45 % cases of CKD (Richard et al. 1994). In another prospective study of 80 patients, prognosis changed in 57 % of patients and therapy changed in 31 % of patients (Turner et al. 1986). Overall, these studies strongly suggest that when in doubt about the cause of the kidney disease, a kidney biopsy should be performed. Figure 2 shows a core of kidney tissue obtained by a native kidney biopsy in a patient with suspected glomerulonephritis. Figure 3 shows the kidney biopsy core under a dissecting microscope, and several glomeruli are seen.

General Biopsy Principles

Renal biopsy may be obtained for a variety of reasons, including establishment of the pathological diagnosis, as an aid to decide optimal therapy or to help determine futility of treatment and to determine the degree of active and chronic changes (Fuiano et al. 2000). In addition, kidney

biopsy can be performed to help assess genetic diseases. Although renal biopsy is not always able to accomplish all these tasks, it remains a valuable diagnostic tool in the following clinical scenarios.

Acute kidney injury (AKI)

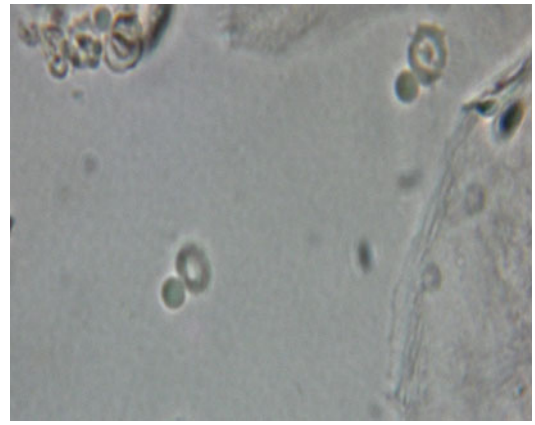
In most cases of AKI, the common causes – prerenal azotemia, acute tubular necrosis, and urinary tract obstruction – can be diagnosed clinically. In a minority of patients, however, a confident diagnosis cannot be made. In these circumstances, a renal biopsy should be performed to aid the clinical diagnosis, especially in the setting of AKI with active urine sediment, or if drug-induced or infection-related kidney diseases are suspected.

Chronic Kidney Disease (CKD)

Renal biopsy can be informative in the patient with unexplained CKD because, in contrast to AKI, it is often difficult to identify the underlying cause on the basis of clinical data alone. Studies have shown that in this setting, the biopsy will demonstrate disease that was not predicted in almost half of cases (Richard et al. 1994). However, in patients with small kidneys bilaterally (<9 cm), the risks of the biopsy might outweigh the diagnostic utility, as these kidneys often show extensive and currently irreversible glomerulosclerosis, tubulointerstitial fibrosis, or both.

Isolated Glomerular Hematuria

Patients with persistent and isolated microscopic hematuria should initially be evaluated for lesions such as kidney stones or genitourinary neoplasm. A glomerular source should be considered if these lesions are not found and particularly if dysmorphic red blood cells (RBCs) known as acanthocytes are present (Figs. 4 and 5). When biopsies are performed in these patients, the histology is often normal or shows IgA nephropathy or thin glomerular basement membrane disease. Because the prognosis for these conditions is excellent in the absence of nephrotic proteinuria, renal impairment, or hypertension, and because specific therapies are not yet available, renal biopsy is not routinely performed for isolated hematuria (Fuiano et al. 2000). However, these patients require close monitoring for the development of proteinuria or renal insufficiency, as this significantly increases the risk of progression to CKD (Szeto et al. 2001).



Indications for Biopsy in Autoimmune GN, Fig. 4 Dysmorphic red blood cells found in the urine of patients with glomerular diseases. Those cells with membrane blebs are called acanthocytes, a subset of dysmorphic RBCs that are fairly specific for glomerular bleeding (Source: Brad Rovin)

Indications for Biopsy in Autoimmune GN, Fig. 5 Acanthocytes (Source: Brad Rovin)

Primary Nephrotic Syndrome

The term nephrotic syndrome is specifically defined by the presence of heavy proteinuria (protein excretion greater than 3.5 g/24 h), hypoalbuminemia (less than 3.0 g/dL), and peripheral edema. Hyperlipidemia and thrombotic disease are also frequently observed.

Most patients with nephrotic syndrome or nephrotic-range proteinuria and no evidence of other systemic disease should be biopsied. One exception may be children aged between 1 year and puberty, as over 90 % will have minimal change disease (Lennon et al. 2010), unless they also have hematuria, positive serology, or renal impairment, or their disease does not respond to empiric corticosteroids. In the absence of a systemic disease (see below), the common differential diagnoses of primary nephrotic syndrome include membranous nephropathy, minimal change disease, or focal segmental glomerulosclerosis (accounting for over 80 % of all cases) (Braden et al. 2000).

Isolated Non-nephrotic Proteinuria

The importance of renal biopsy in patients with non-nephrotic proteinuria is controversial. A renal biopsy generally is not performed in a patient presenting with low-grade proteinuria (less than 500–1,000 mg/day) in the absence of glomerular hematuria, abnormal renal function, and clinical or serologic evidence of a systemic disease. Many nephrologists routinely perform a renal biopsy in patients with somewhat higher degrees of non-nephrotic proteinuria (1–2 g/day), except in settings where this may be explained by conditions such as long-standing diabetes mellitus or hypertension.

Renal Dysfunction Associated with Systemic Diseases

A number of systemic diseases may affect the kidney, and sometimes a kidney biopsy is the best way to diagnose a systemic disease. Alternatively, a systemic disease may be presumed by clinical characteristics or specific serologic tests, but the kidney biopsy is required to determine prognosis. Treatment often entails the treatment of the primary disease, but kidney involvement may require additional or more intense therapies. Common systemic disorders involving the kidney are:

Diabetes Mellitus: Patients with diabetes mellitus and renal dysfunction do not usually require a biopsy if the clinical setting is compatible with diabetic nephropathy (diabetes of long duration, evidence of other microvascular diabetic complications, isolated proteinuria). However, in patients with an atypical presentation (proteinuria associated with glomerular hematuria, absence of retinopathy or neuropathy, rapid onset of proteinuria <5 years from documented onset of diabetes, rapid decline in renal function, the presence of immunologic abnormalities), a renal biopsy should be performed.

Antineutrophil Cytoplasmic Antibodies (ANCA)-Associated Vasculitis and Anti-glomerular Basement Membrane (Anti-GBM) Antibody Disease: Serologic testing for ANCA and for anti-GBM antibodies helps support a diagnosis of renal small-vessel vasculitis or Goodpasture's disease. However, a renal biopsy should still be performed to confirm the diagnosis and to clarify the extent of active inflammation versus chronic fibrosis. This information can be crucial not only in making decisions about the immunosuppressive therapy but can also give an idea about potential for recovery.

Systemic Lupus Erythematosus (SLE): Renal involvement occurs in approximately 60 % of patients with SLE (Beck and Salant 2009). There are multiple histological subtypes of lupus nephritis (LN) and the optimal treatment varies with the subtype (Waldman and Appel 2006). Although lupus nephritis can be identified by noninvasive criteria (autoantibodies, urine protein excretion, renal function, and urine sediment abnormalities), a renal biopsy elucidates the histological subtype of LN, the level of acute activity, and the extent of chronic fibrosis, thereby providing robust guidance for immunosuppressive therapy (Faurschou et al. 2006).

Miscellaneous: Other systemic diseases, such as amyloidosis, sarcoidosis, and myeloma, can be diagnosed with a renal biopsy. However, because these diagnoses can often be made by other approaches, a renal biopsy is indicated only if the diagnosis remains in doubt or when the knowledge of renal involvement might alter the therapy.

Renal Transplant Dysfunction

Renal allograft dysfunction requires a renal biopsy to determine the cause, particularly in the absence of other identifiable etiology such

as ureteral obstruction, urinary sepsis, or renal artery stenosis (Colvin 2007). In the early posttransplantation period, this is most useful in differentiating acute rejection from acute tubular necrosis (Solez et al. 2008). Later, renal biopsy can differentiate late acute rejection from chronic allograft nephropathy, recurrent or de novo glomerulonephritis, calcineurin inhibitor toxicity, and BK nephropathy.

Familial Renal Disease

A renal biopsy can be helpful in the investigation of patients with a family history of renal disease, and a biopsy performed on one affected family member may secure the diagnosis for the whole family.

Limitations of the Kidney Biopsy

It is important to recognize that prognostication based on renal pathology alone may be affected by the sample size and may not be very accurate in biopsies with few glomeruli (i.e., $\leq$5). Sampling errors can occur particularly if the disease is focal in nature, such as in renal sarcoidosis (Shah et al. 2011). The findings in renal biopsy always need to be interpreted in the context of the clinical and laboratory features. Chronic changes (interstitial fibrosis and tubular atrophy), for example, are a sign of the magnitude and duration of prior injury. The final decision to conduct a biopsy should, in most cases, be based on whether or not the biopsy will change the therapeutic plan (Toto 2009).

Because the kidney biopsy is an invasive procedure, it is generally not repeated on a serial basis to evaluate the effect of treatment on kidney injury. Treatments are followed with clinical markers such as changes in the urine sediment, level of proteinuria, and kidney function. None of these clinical parameters are sensitive or specific enough to be surrogates of kidney pathology. However, ongoing studies using urine proteomics are beginning to find biomarkers that accurately reflect renal pathology (Bramham et al. 2009; Zhang et al. 2012; Brunner et al. 2012). The goal of these studies is to develop urine biomarker panels that can be used to noninvasively follow the effects of treatment on renal pathology over time.

The Future of the Kidney Biopsy

Given the advances in molecular diagnoses through a multitude of "omics" platforms, it is anticipated that expanded analyses of kidney biopsies, beyond light, immunofluorescence, and electron microscopy, will be used to understand the molecular pathogenesis of kidney diseases (Satoskar et al. in press; Sarwal et al. 2003; Peterson et al. 2004). Examination of the glomerular genome, proteome, or transcriptosome will distinguish differences between phenotypically similar renal lesions (Sarwal et al. 2003; Peterson et al. 2004). This information can then be used to more precisely classify an individual's disease and personalize therapy by targeting pathways activated in that individual (Rovin et al. 2009; Hodgin et al. 2010; Yasuda et al. 2006).

Cross-References

- ▶ Anti-glomerular Basement Membrane Disease
- ▶ IgA Nephropathy
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Sarcoidosis
- ▶ Spectrum of Minimal Change Disease to Focal Segmental Glomerulosclerosis
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Vasculitis and the Kidney
- ▶ Vasculitis: Granulomatosis with Polyangiitis (Wegener's)

References

Appel GB. Renal biopsy: How effective, what technique, and how safe. J Nephrol. 1993;6:4–9.

Beck Jr LH, Salant DJ. Treatment of membranous lupus nephritis: where are we now? J Am Soc Nephrol. 2009;20(4):690–1.

Braden GL, Mulhern JG, O'shea MH, Nash SV, Ucci AA, Germain MJ. Changing incidence of glomerular diseases in adults. Am J Kidney Dis. 2000;35(5):878–83.
Bramham K, Mistry HD, et al. The non-invasive biopsy – will urinary proteomics make the renal tissue biopsy redundant? QJM Int J Med. 2009;102:523–38.
Briganti EM, Dowling J, et al. The incidence of biopsy-proven glomerulonephritis in Australia. Nephrol Dial Transplant. 2001;16(7):1364.
Brunner HI, Bennett MR, Mina R, Suzuki M, Petri M, Kiani AN, et al. Non-invasive renal protein biomarkers are associated with histological features of lupus nephritis. Arthritis & Rheumatism; doi: 10.1002/art.34426 (Accepted: Feb 02, 2012).
Cohen AH, Nest CC, et al. Clinical utility of kidney biopsies in the diagnosis and management of renal disease. Am J Nephrol. 1989;9(4):309–15.
Colvin RB. Antibody-mediated renal allograft rejection: Diagnosis and pathogenesis. J Am Soc Nephrol. 2007;18:1046–56.
Faurschou M, Starklint H, Halberg P, Jacobsen S. Prognostic factors in lupus nephritis: diagnostic and therapeutic delay increases the risk of terminal renal failure. J Rheumatol. 2006;33:1563–9.
Fuiano G, Mazza G, Comi N, et al. Current indications for renal biopsy: a questionnaire-based survey. Am J Kidney Dis. 2000;35:448–57.
Hodgin JB, Borczuk AC, Nasr SH, Markowitz GS, Nair V, Martini S, et al. A molecular profile of focal segmental glomerulosclerosis from formalin-fixed, paraffin-embedded tissue. Am J Pathol. 2010;177(4):1674–86.
Iversen P, Brun C. Aspiration biopsy of the kidney. J Am Soc Nephrol. 1997;8:1778–87.
Kark RM, Muehrcke RC. Biopsy of kidney in prone position. Lancet. 1954;266:1047–9.
Lennon R, Watson L, et al. Nephrotic syndrome in children. Paediatr Child Health. 2010;20(1):36–42.
Peterson KS, Huang JF, Zhu J, D'Agati V, Liu X, Miller N, et al. Characterization of heterogeneity in the molecular pathogenesis of lupus nephritis from transcriptional profiles of laser-captured glomeruli. J Clin Invest. 2004;113(12):1722–33.
Richard NT, Darby S, et al. Knowledge of renal histology alters patient management in over 40 % of cases. Nephrol Dial Transplant. 1994;9(9):1255–9.
Rovin BH, Mckinley AM, Birmingham DJ. Can we personalize treatment for kidney diseases? Clin J Am Soc Nephrol. 2009;4(10):1670–6.
Sarwal M, Chua M, Kambham N, Hsieh S, Satterwhite T, Masek M, et al. Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. N Engl J Med. 2003;349(2):125–38.
Satoskar A, Shapiro J, Bott C, Song H, Nadasdy G, Brodsky S, et al. Characterization of glomerular diseases using proteomic analysis of laser capture microdissected glomeruli. Modern Pathol; 2012;25:709–721.
Shah R, Shidham G, et al. Diagnostic utility of kidney biopsy in patients with sarcoidosis and acute kidney injury. Int J Nephrol Renovascular Dis. 2011;4:131–6.
Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant. 2008;8:753–60.
Szeto CC, Lai FM, To KF, et al. The natural history of immunoglobulin a nephropathy among patients with hematuria and minimal proteinuria. Am J Med. 2001;110:434–7.
Topham PS, Chen Y. Renal biopsy. In: Floege J, Johnson RJ, Feehally J, editors. Comprehensive clinical nephrology. 4th ed. Philadelphia: Mosby; 2010. p. 75–82.
Toto RD. Renal biopsy: The nephrologist's view point. In Xin JZ, Zoltan L, Tibor N, Vivette D. Fréd & Silva's diagnostic renal pathology (First edition). Cambridge University Press: New York, NY USA, p. 47–55.
Turner MW, Hutchinson TA, et al. A prospective study on impact of renal biopsy in clinical management. Clin Nephrol. 1986;26(5):217–21.
Waldman M, Appel GB. Update on the treatment of lupus nephritis. Kidney Int. 2006;70:1403–12.
Yasuda Y, Cohen CD, Henger A, Kretzler M. Gene expression profiling analysis in nephrology: towards molecular definition of renal disease. Clin Exp Nephrol. 2006;10(2):91–8.
Zhang X, Nagaraja HN, Nadasdy T, Song H, McKinley A, Prosek J, et al. A composite urine biomarker of interstitial inflammation in lupus nephritis kidney biopsies. Kidney Int. 2012;81(4):401–6.

Inflammatory Bowel Disease

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Synonyms

Crohn's disease; IBD; Ulcerative colitis

Definition

The inflammatory bowel diseases (IBDs) include Crohn's disease (CD) and ulcerative colitis (UC) and are chronic diseases characterized by relapsing inflammation in the intestinal tract.

Epidemiology

The prevalence of IBD in North America has been reported to be between 44–201/100,000 (CD) and 37–238/100,000 (UC) (Cosnes et al. 2011). However, the number of people diagnosed with IBD is increasing, particularly in countries such as Asia and India where the incidence has historically been low (Molodecky et al. 2011). The peak age of onset of IBD occurs between 20 and 40 years of age. Smoking is a significant risk factor for the development of CD but may be protective in patients with UC (Mahid et al. 2006). Appendectomy before the age of 20 years appears to protect against development of UC (Koutroubakis et al. 2002).

Clinical and Pathologic Features of Ulcerative Colitis

Patients with UC typically present with symptoms of bloody diarrhea and abdominal cramping. Diarrhea may be absent in patients with disease limited to the rectum (proctitis), and symptoms may arise insidiously in these patients. Severe disease may be accompanied by fevers, abdominal pain or abdominal distension, anemia, elevated white blood cell count, and inflammatory markers (erythrocyte sedimentation rate and C-reactive protein). Patients with severe colitis involving the entire colon (pancolitis) are at risk for developing colonic dilation and impaired motility termed toxic megacolon. While the exact mechanism underlying toxic megacolon is unknown, increased inducible nitric oxide synthase has been found in the colonic wall of patients with toxic megacolon (Mourelle et al. 1995). These patients are at high risk for perforation of the colon and may require urgent surgery.

The diagnosis of UC is made by colonoscopy with endoscopic biopsies. The endoscopic appearance is characterized by diffuse mucosal erythema and ulceration with loss of vascular markings due to mucosal edema. In addition, there may be friability of the mucosa with contact or spontaneous bleeding, and exudates may be present. In severe ulcerative colitis, deep ulcers may be encountered. Inflammation always involves the rectum and extends towards the cecum in a continuous diffuse pattern. In one-third of patients, disease is limited to the rectum, while an additional one-third have colitis proximal to the splenic flexure. One quarter of patients have pancolitis (Moum et al. 1999). In patients with severe pancolitis, the adjacent ileum may be inflamed in a process called backwash ileitis.

The histopathology of the colon in UC is characterized by inflammation limited to the mucosa and superficial submucosa of the colon. In active disease, increased neutrophilic and mast cell infiltrates are present in the lamina propria and may cause crypt abscess (neutrophils within the crypt lumen). Chronic inflammatory infiltrate with lymphocytes, plasma cells, and eosinophils may be present in the lamina propria. Evidence of chronicity is a diagnostic feature of IBD and is characterized by crypt distortion and branching, crypt atrophy, and Paneth cell metaplasia. Granulomas are not seen in ulcerative colitis.

Clinical and Pathologic Features of Crohn's Disease

Crohn's disease may have a varied clinical presentation depending on the site and nature of the disease involvement. Nonspecific symptoms can delay the time to diagnosis in patients with CD (Pimentel et al. 2000). Symptoms occur when disease causes pain, systemic inflammation including extraintestinal manifestations (see Extraintestinal Manifestations in IBD below), diarrhea, malabsorption, nutritional deficiency, or weight loss. Diarrhea is often non-bloody and less severe than that seen in UC. Alternatively, patients may present with symptoms when transmural inflammation leads to stricture or penetration causing fistula or abscess. Colonic Crohn's disease typically causes diarrhea with or without rectal bleeding.

Unlike UC where inflammation is limited to the rectum and extends upwards to involve the

entire colon, inflammation in Crohn's disease can involve any part of the intestinal tract, most commonly the ileum and colon. Ten to fifteen percent of patients will have upper gastrointestinal involvement of the esophagus, stomach, or duodenum (Cosnes et al. 2011). Disease location tends to be stable over time (Louis et al. 2001). Inflammation in CD is transmural, and this can result in complications over time including penetrating disease or strictures. Almost one-half of patients with Crohn's disease will develop perianal complications including perianal abscess, fistula, stenosis, or fissures (Schwartz et al. 2001, 2002; Cosnes et al. 2008).

CD is typically diagnosed by tissue biopsy during esophago-gastroduodenoscopy or colonoscopy. Imaging techniques such as CT, MRI, small bowel follow through, and capsule endoscopy can be helpful at defining the location and extent of disease. The endoscopic appearance of CD is characterized by discontinuous deep ulcerations of the intestinal mucosa often called cobblestoning or can be limited to aphthous ulcerations of the small bowel and colon. Large areas of the intestine may be uninvolved, and these have been termed "skip" areas.

The histopathology of CD is characterized by inflammation that may involve any layer of the intestinal wall from the mucosa to the serosa. Early in the disease, pathology in the colon may be identical to UC with neutrophilic and lymphoplasmacytic infiltrate and crypt architectural distortion. However, with time, the pathologic picture is marked by T lymphocyte and macrophage infiltration. Noncaseating granulomas are present in about 30 % of biopsies in Crohn's disease (Heresbach et al. 2005), but are not present in ulcerative colitis. In some cases, ulcerative colitis may be difficult to distinguish clinically and histologically from Crohn's colitis, and this has been referred to as indeterminate colitis.

Extraintestinal Manifestations of IBD

Patients with IBD may develop inflammation in organs outside the intestines. The most common extraintestinal manifestation of IBD is arthropathy with a prevalence of 10–35 % (Larsen et al. 2010). Joint manifestations can include sacroiliitis or polyarticular arthritis of the large joints. Recurrent aphthous ulcers of the mouth occur in up to 20 % of patients with IBD. Skin manifestations associated with IBD include pyoderma gangrenosum and erythema nodosum. Ocular inflammation including iritis, episcleritis, and uveitis occurs with increased frequency in patients with IBD.

The aforementioned symptoms generally mirror intestinal symptoms, and often treatment directed towards intestinal inflammation leads to improvement in extraintestinal manifestations. However, in the case of sacroiliitis, uveitis, and pyoderma gangrenosum, the course may progress independent of intestinal inflammation. Patients with IBD are also at increased risk for development of Primary Sclerosing Cholangitis (PSC), an inflammatory disorder of the bile ducts that leads to scarring and recurrent cholangitis. The disease course of PSC progresses independent of intestinal activity.

Other non-intestinal manifestations may occur as a complication of intestinal disease or treatment. These include development of gallstones (secondary to fat or bile acid malabsorption), kidney stones (exacerbated by dehydration and excess oxalate absorption), and osteoporosis (secondary to calcium and vitamin D deficiency and exacerbated by low body mass index and steroid use).

IBD Pathogenesis

Three specific factors contribute to the initiation and perpetuation of the inflammatory process in IBD. These include the host genetics, intestinal microbial factors, and the response of the mucosal immune system.

Genetics of IBD

Twin studies provided initial evidence that there is a strong genetic contribution to the development of IBD. In Crohn's disease, the effects appear to be more pronounced with an estimated

50 % genetic contribution versus 20 % in ulcerative colitis (Kaser and Blumberg 2011). While a very rare autosomal recessive form of early IBD has been found to result from defects in IL-10 and the IL-10 receptor genes, most genes associated with IBD are common, low penetrance variants. Of these, NOD2 mutations have the highest contribution to CD with homozygous mutations conferring a 20-fold increased risk. However, most other gene associations confer less than a 1.5-fold risk (Cho and Brant 2011).

Genome-wide association studies (GWAS) recently identified over 100 genetic loci associated with IBD. The precise functional allele or causative genes for the majority of these loci have not been defined, but candidate genes have been identified that highlight novel pathways for investigation. These pathways include genes involved in microbe sensing and recognition (NOD2), autophagy (ATG16L1, IRGM, LRRK2), lymphocyte activation, function and regulation (HLA Class II, IL-10, IL-2RA, IL-23R, IL12B, JAK2, STAT3, SMAD3, ICOS, PTPN22), endoplasmic reticulum stress responses (Xbp1), and intestinal barrier function (MUC1, MUC19, ARPc, CDH, OCTN1/2, LAMB1). There is significant overlap between genes found to be associated with CD and UC. However, unique genetic associations with each disease are present and suggest differences in disease pathogenesis. In addition to overlap within CD and UC, genetic polymorphisms associated with IBD are shared with other autoimmune diseases including PSC, type I diabetes, psoriasis, lupus, multiple sclerosis, and ankylosing spondylitis.

Role of the Intestinal Microbiota

Substantial evidence suggests that the intestinal microbiota play a significant role in initiating and/or perpetuating the intestinal inflammation in IBD. In animal models of IBD, colitis does not develop under germ-free conditions (Round et al. 2009). In humans, it has been demonstrated that diversion of the fecal stream after surgery for Crohn's disease ameliorates inflammation; however, disease recurs quickly once the fecal stream is reconstituted (Rutgeerts et al. 1991). Additionally, antibiotic therapy has been shown to be effective as a treatment for both Crohn's disease and pouchitis. Defects in tolerance to commensal bacteria, an altered balance of bacterial populations and host-environmental and genetic factors that impact bacterial sensing and clearance, have been implicated in development of IBD.

Defects in Tolerance

Patients with IBD and their family members have defects in the establishment of oral tolerance, the phenomenon by which antigens administered via the oral route lead to suppression of the systemic immune response upon subsequent challenge (Kraus et al. 2004). A percentage of patients with IBD have serum antibodies against endogenous microflora, suggesting a similar lack of tolerance to commensal flora. Antibodies identified at a higher rate in patients with IBD include anti-Saccharomyces cerevisiae antibodies (ASCA), anti-E. coli outer membrane porin C (OmpC), anti-bacterial flagellin (cBir1), and anti-Pseudomonas fluorescens 12(12).

Altered Microbial Populations

In addition to a loss of tolerance to commensal bacteria, studies have demonstrated that the composition of commensal bacterial flora is significantly altered in patients with IBD. However, no specific causative microbe has been identified. This IBD-specific microbial signature is characterized by reduced bacterial diversity (Frank et al. 2007, 2008, 2010; Qin et al. 2010). There is a relative reduction in Bacteroidetes and clostridial firmicute species. Among the groups of bacteria depleted in patients with IBD are symbiont bacteria that have been shown to have anti-inflammatory properties. For example, *Bacteroides fragilis* produces an immunomodulatory molecule, Polysaccharide A (PSA), which induces FoxP3+ regulatory T cells (Round et al. 2010). Another bacteria that appears to be selectively depleted in Crohn's disease is *Faecalibacterium prausnitzii*. *F. prausnitzii* secretes soluble factors that inhibit NF-$_{\kappa}$B activation and increase IL-10 production. Patients undergoing resection for Crohn's disease

with low numbers of this organism have been shown to be at increased risk for early recurrence of disease (Sokol et al. 2009). In addition to depletion of protective species, patients with IBD have higher numbers of mucosa-associated bacteria, particularly invasive E. coli species. Whether these changes in the content and diversity of bacterial flora in IBD occur as a secondary response to inflammation or constitute a primary event remains an issue of debate.

Defects in Bacterial Sensing and Elimination

Genetic defects resulting in the inability to sense or eliminate bacteria may also contribute to increased mucosa-associated bacteria in Crohn's disease. Mutations in the bacterial sensing protein, NOD2, are significantly associated with ileal and stricturing Crohn's disease. NOD2 is expressed in both hematopoietic and epithelial cells and responds to the bacterial product muramyl dipeptide (MDP) leading to the activation of $NF_{\kappa}B$ and MAP kinase signaling. Primary cells isolated from patients homozygous or heterozygous for NOD2 mutations have defects in $NF_{\kappa}B$ activation in response to MDP (Abraham et al. 2006). Mice lacking NOD2 do not develop spontaneous intestinal inflammation but have been shown to express reduced antimicrobial defensins and demonstrate defects in efficient bacterial clearance (Kobayashi et al. 2005).

Studies of the autophagy genes associated with IBD (ATG16L1, IRGM, LRRK2) have highlighted the importance of this pathway in mucosal homeostasis in response to microorganisms. Autophagy is the process by which cytosolic contents are engulfed via a double-layered membrane and targeted via fusion for lysosomal degradation. Degraded cellular contents can be presented by MHC Class II molecules or be recognized by Toll-like receptors (TLRs). Autophagy is important for the removal of damaged organelles, generation of energy by recycling of proteins, and removal of intracellular microorganisms. Macrophages derived from ATG16L1-deficient mice demonstrate enhanced IL-1β and IL-18 response to stimulation with lipopolysaccharide (LPS) and TNFα (Saito et al. 2008), suggesting that autophagy regulates the inflammatory response to bacterial endotoxin. Recent studies have also highlighted a role for ATG16L1 in altered response to intestinal viral infections. Mice expressing hypomorphic Atg16L1 have been found to have abnormal Paneth cell morphology and function (Cadwell et al. 2008). Paneth cells are epithelial cells found at the base of intestinal crypts that secrete antimicrobial peptides. Interestingly, the Paneth cell abnormalities in these mice are dependent on persistent infection with a specific mouse Norovirus strain. While mice did not develop spontaneous intestinal inflammation, when challenged with dextran sulfate sodium (DSS) in the drinking water, they exhibited increased severity with transmural ileocolonic inflammation that was responsive to antibiotics (Cadwell et al. 2010). Paneth cell morphologic abnormalities and defects in antimicrobial peptide secretion have similarly been observed in CD patients with the ATG16L1 risk allele (Cadwell et al. 2008) and in patients with NOD2 mutant alleles. These data show that in the right genetic context, an infectious trigger can lead to alterations in host defenses that result in inappropriate immune responses to the intestinal microbiota.

The Mucosal Immune Response in IBD

Defects in Immune Regulation

In health, the LP contains a large number of activated immune cells. However, inflammation is actively restrained through multiple mechanisms including regulatory T cells and secretion of immunoregulatory cytokines (TGFβ and IL-10). It is postulated that a breakdown in homeostatic immune regulation may contribute to the development of IBD. A number of subsets of regulatory T cells that can inhibit T cell effector function have been described to be active in the intestinal mucosa and include Tr1 and Th3 cells, the natural and induced CD4+FoxP3+ Tregs, and CD8+ Tregs. These populations inhibit the immune response through the secretion of the anti-inflammatory cytokines IL-10 and TGFβ and/or inhibit by contact-dependent mechanisms.

While defects in FoxP3+ Tregs have been described in many animal models of colitis

CD4+FoxP3+, T cells are increased in number in the inflamed intestines in patients with IBD compared to healthy controls (Makita et al. 2004; Maul et al. 2005; Holmen et al. 2006) and have normal suppressive function in vitro. Despite increased numbers and normal function, they are unable to suppress inflammation in IBD. It has been proposed that there may be a relative deficiency of Tregs compared to other inflammatory diseases. It is also possible that there are local environmental factors within the LP that render Tregs ineffective in vivo, while able to function ex vivo. Alternatively, T effectors in the LP of patients with IBD may be resistant to Treg-mediated suppression. This has been suggested by data demonstrating T effector resistance associated with increased expression of the TGFβ inhibitory protein, SMAD7, in LP T cells from patients with IBD (Fantini et al. 2009). Lastly, it has recently been demonstrated that the lamina propria of patients with Crohn's disease contains a subset FoxP3+ T cells that co-express IL-17 (Hovhannisyan et al. 2011). These data suggest that the Tregs are able to acquire an inflammatory phenotype that may perpetuate disease in the microenvironment of the inflamed intestine.

It is possible that alternative Treg subsets, such as CD8+ Tregs, play a dominant role in maintaining intestinal homeostasis. CD1d-restricted CD8+ Tregs can be generated by the interaction with normal intestinal epithelium and are also isolated from the LP of normal individuals (Allez et al. 2002). These Tregs are able to suppress T effector function in a contact-dependent manner. However, CD8+ T cells isolated from patients with Crohn's disease exhibit a reduction in suppressor function that may contribute to uncontrolled inflammation (Rabinowitz et al. 2013).

IL-10 and TGFβ are immunoregulatory cytokines that have been implicated in regulation by both FoxP3+ and FoxP3-negative regulatory cells (Tr1 and Th3). The importance of these cytokines in the maintenance of intestinal homeostasis has been clearly demonstrated in animal models, where deficiency in IL-10 results in spontaneous colitis and deficiency in TGFβ1 in FoxP3+ Tregs results in inability to control colitis (Li et al. 2007). Evidence that alterations in these cytokine pathways might contribute to human IBD has been supported by recent GWAS demonstrating association with IL-10, IL-10 receptor, and SMAD3 polymorphisms.

Role of Inflammatory Cytokines

Crohn's disease and ulcerative colitis are characterized by distinct patterns of cytokine expression in the lamina propria (LP). Crohn's disease was initially described to be a Th1-mediated disease, characterized by the expression of large amounts of IFN_{γ}, TNF_{α}, and IL-12 (Fuss et al. 2006). In contrast, LP from UC patients demonstrates increased secretion of IL-5 and IL-13 (Fuss et al. 1996, 2004). Others have shown there is also an increased production of IFN_{γ} and TNF_{α} in the LP of UC patients. While UC was initially described to be a Th2-mediated disease, it probably derives input from several different T cell subsets.

More recently, it has been recognized that IL-17 is also secreted by LP T cells in both CD and to a lesser extent in UC (Fujino et al. 2003; Nielson et al. 2003; Kobayashi et al. 2008; Sakuru et al. 2009). IL-23 maintains and expands IL-17-expressing T cells. Association of genes involved in the IL-23 signaling pathway identified by GWAS in CD and UC has cemented a role for the Th17 cytokines in IBD. GWAS have identified IL-23 receptor mutations as protective for both CD and UC. In addition, IL-12β (p40), the shared subunit of IL-12 and IL-23, and downstream signaling molecules JAK2 and STAT3 have also been associated with IBD. Peripheral blood from patients with CD have increased number of IL-23 receptor-bearing cells and respond to IL-23 with more robust IL-17 and IFN_{γ} production (Kleinshack et al. 2009) suggesting that IL-23 may drive inflammation in CD. Supportive of a role for IL-23 in IBD, in a mouse model of colitis that results from transfer of effector T cells into immunodeficient mice, a lack of IL-23 receptor on T cells resulted in protection from colitis. In this model, IL-23 appeared to be a negative regulator of FoxP3 induction in the colon such that T cell transfer into IL-23-deficient immunodeficient

mice resulted in increased numbers of colonic FoxP3+ regulatory T cells (Iscue et al. 2008). It is unclear whether IL-23 exerts its effects in IBD via restraint of regulatory T cell induction or an enhancement of IL-17-producing T cells.

The proinflammatory cytokine, TNF_{α}, is elevated in tissue and blood of patients with both UC and CD and is secreted as a secondary response to tissue inflammation by innate immune cells. TNF_{α} activates $NF_{\kappa}B$ and mitogen-activated protein (MAP) kinases and perpetuates inflammation through increased production of proinflammatory cytokines IL-1β, IL-6, and IL-12; recruitment of inflammatory cells; initiation of acute phase response; and inhibition of apoptosis. The success of anti-TNF_{α} agents in the treatment of IBD has demonstrated a clear role for this cytokine in its pathogenesis.

Medical Therapy for Inflammatory Bowel Disease

There is significant overlap in medical therapy for UC and Crohn's disease. The medical context of therapy is important as certain medications are effective at inducing remission, while others prevent disease relapse. Specific medications may be better suited to treat extraintestinal manifestations of IBD and fistulizing Crohn's disease. Recent attention has also been given to those medications that may prevent relapse after surgery in Crohn's disease.

Aminosalicylates (Sulfasalazine, Mesalamine)

Mechanism of Action: 5-ASA agents block both cyclooxygenase and lipoxygenase pathways reducing prostaglandin and leukotriene synthesis (Sharon et al. 1978; Ligumksy et al. 1981; Hawkey et al. 1985). They can act as free radical scavengers and minimize tissue damage (Ahnfelt-Ronne et al. 1990). 5-ASAs also inhibit T cell activation and IL-2 production (Stevens et al. 1995) and can directly inhibit TNF_{α} (Shanahan et al. 1990). 5-ASAs may also exert effects through activation of peroxisome proliferator-activated receptor-gamma (PPAR-gamma), a nuclear receptor critical for maintenance of colonic antimicrobial peptides called beta-defensins (Rousseaux et al. 2005; Peyrin-Biroulet et al. 2010).

Ulcerative Colitis: The aminosalicylate (5-ASA) medications are first-line therapy for induction and maintenance therapy in mild to moderate UC. Dosing of 5-ASAs may be important in patients with moderate disease activity where higher doses may improve response rates (Hanauer et al. 2005). Topical mesalamine can be used to induce and maintain remission in patients with distal UC (Cohen et al. 2000; Marshall et al. 2000).

Crohn's Disease: While studies have clearly shown the benefit of 5-ASAs in the maintenance and remission of UC, the benefit in Crohn's disease is unclear. Two recent meta-analyses showed no benefit for mesalamine over placebo in inducing remission in CD. One of these studies showed a small benefit of sulfasalazine over placebo in induction of remission, while the other study demonstrated a trend towards benefit. The benefits of sulfasalazine appeared to be greatest in patients with colitis. No significant benefit was seen for 5-ASAs for maintenance of remission in CD (Lim et al. 2010; Ford et al. 2011).

Corticosteroids

Mechanism of Action: Corticosteroids bind to glucocorticoid receptors expressed in multiple cell types and lead to transcription of a gene program with diverse effects. Corticosteroid effects include inhibition of prostaglandin synthesis, blockade of MAP kinase signaling pathways, and $NF_{\kappa}B$ transcription (Rhen et al. 2005).

Ulcerative Colitis: Corticosteroids are effective at inducing remission in ulcerative colitis and are used in patients with moderate to severe symptoms who have not responded to 5-ASAs. In patients not responding to prednisone, intravenous steroids may be effective. Patients with symptoms that have not improved after 3–5 days of intravenous steroids are unlikely to respond, and an alternative medical or surgical strategy should be initiated. Systemic steroids are not used in the maintenance of remission for

ulcerative colitis. Topical steroid preparations are effective for induction of remission in distal ulcerative colitis (Mulder et al. 1996).

Crohn's Disease: Steroids have been demonstrated to induce remission in Crohn's disease. However, a population study in Olmstead county revealed 28 % of patients with CD could not be tapered off steroids by a year (Faubion et al. 2001), arguing that alternatives to steroids should be sought in this disease. Budesonide is a nonsystemic steroid preparation that delivers glucocorticoid to the terminal ileum and right colon with high first-pass metabolism in the liver, resulting in fewer systemic side effects. Budesonide is as effective as systemic steroids in patients with ileal or ileo-right colonic disease (Rutgeerts et al. 1994; Gross et al. 1996; Bar-Mier et al. 1998; Campieri et al. 1997).

In doses less than 6 mg/day, randomized studies have not shown a benefit of budesonide in maintenance of remission (Papi et al. 2000; Simms and Steinhart 2001).

Antibiotics

Mechanism of Action: Although a causative organism has not been identified in patients with IBD, evidence supports a role for an aberrant response to commensal bacteria, and antibiotics have been used in its treatment.

Data regarding the effectiveness of antibiotics in Crohn's disease and ulcerative colitis has been conflicting and difficult to interpret due to heterogeneity in antibiotic type, combination, dosing, and duration. A meta-analysis looking at antibiotic therapy for the induction and remission of UC and CD revealed a modest effect for the treatment of active CD and UC and maintenance of remission in CD. However, larger studies of individual antibiotic regimens are necessary (Kahn et al. 2011).

Immunomodulators (6MP/Azathioprine)

Mechanism of Action: The immunomodulator class of drugs, including 6MP and its prodrug azathioprine (AZA), are purine analogues. These drugs are converted into their metabolites 6-thioguanine (6-TG), 6-methyl-mercaptopurine (6MMP), and 6-thiouric acid and can become incorporated into DNA and interfere with nucleic acid synthesis. In addition, 6MMP has been shown to inhibit de novo purine synthesis (Maltzman et al. 2003). Azathioprine has also been shown to induce apoptosis in CD4+ T cells (Tiede et al. 2003).

Ulcerative Colitis: Studies of AZA withdrawal have demonstrated a higher rate of relapse in patients withdrawn to placebo versus those continued on AZA. A study of steroid-dependent UC patients randomized to receive mesalamine 3.2 g/daily or AZA 2 mg/kg daily demonstrated increased endoscopic and clinical remission and steroid tapering in the group randomized to AZA. Based on these studies immunomodulators are primarily used for steroid sparing and maintenance of remission in UC.

Crohn's Disease: A number of studies have been performed evaluating immunomodulators for the treatment Crohn's disease and have been reviewed in a meta-analysis of trials looking at induction (Sandborn et al. 2000) and maintenance of remission (Pearson et al. 2000). Immunomodulators were found to be effective at both induction and maintenance of remission for CD. However, the maximal clinical benefit of these medications is seen after 3–4 months, generally necessitating the use of another induction agent when starting these medications.

Methotrexate

Mechanism of Action: Methotrexate is a folate analog that inhibits folate-dependent enzymes important for purine and pyrimidine synthesis and inhibits cell division of rapidly dividing cells including lymphocytes. Methotrexate may also control lymphocyte function by inducing apoptosis of activated CD4+ T cells (Nielson et al. 2007). In addition, methotrexate inhibits 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase, an enzyme involved in de novo purine synthesis. In the absence of AICAR transformylase, increased AICAR leads to elevations in both intra- and extracellular adenosine (Krener et al. 2004). Adenosine has anti-inflammatory properties including inhibition of neutrophil adherence

(Cronstein et al. 1991) and inhibition of cytokine response by hematopoietic cells and has been implicated in regulatory T cell (Treg) function (Ernst et al. 2010). Studies in both animals and humans have suggested that adenosine antagonists can partially inhibit the effects of methotrexate in vivo (Montesinos et al. 2000; Nesher et al. 2003).

Crohn's Disease: Intramuscular methotrexate has been shown to induce and maintain remission in Crohn's disease (Feagan et al. 1995, 2000).

Ulcerative Colitis: Randomized controlled trials using oral methotrexate in ulcerative colitis failed to demonstrate benefit in induction or maintenance of remission (Oren et al. 1996; Mat-Jimenez et al. 2000). Studies utilizing parenteral dosing effective in Crohn's disease have not been evaluated in UC to date.

Cyclosporin

Mechanism of Action: Cyclosporin (CSA) binds cyclophilin and blocks the phosphatase activity of calcineurin. Calcineurin blockade prevents nuclear translocation of the transcription factor NFATc and downstream transcription of genes critical for T cell activation including production of IL-2 (Ho et al. 1996).

Ulcerative Colitis: In randomized controlled trials studying patients with IV steroid-refractory UC, CSA induces response in 82 % of patients (Lichtiger et al. 1994). Retrospective studies have reported long-term colectomy-free survival at 16–78 months in 40–90 % of patients transitioned to oral CSA transitioned to immunomodulator therapy (Fernandez-Banares et al. 1996; Cohen et al. 1999; Campbell et al. 2003; Message et al. 2005).

Crohn's Disease: Randomized studies of oral CSA for the treatment of CD have not consistently demonstrated benefit (McDonald et al. 2005). Intravenous dosing analogous to treatment for UC has not been tested in CD.

Anti-TNF Agents (Infliximab, Adalimumab, Certolizumab)

Mechanism of Action: TNF_α is a proinflammatory cytokine that perpetuates tissue damage IBD. Specific TNF_α blockade in IBD has been accomplished through the development of anti-TNF antibodies (infliximab and adalimumab) and an anti-TNF F(ab)' fragment linked to polyethylene glycol (Certolizumab). In addition to neutralization of soluble TNF_α, infliximab and adalimumab are capable of binding to cells expressing transmembrane-bound TNF_α. These antibodies can fix complement and allow for antibody-mediated cytotoxicity of TNF_α-bearing cells. In addition, binding of transmembrane TNF_α has been reported to induce reverse signaling in monocytes and lead to apoptosis, cell cycle arrest, and IL-10 production (Mitoma et al. 2005). Induction of apoptosis has been observed in the intestinal mucosa of patients undergoing infliximab therapy (Ten Hove et al. 2002; Van Den Brande et al. 2007). However, induction of apoptosis is not necessarily a critical mechanism of anti-TNF therapy, as certolizumab is not capable of inducing apoptosis in vitro and is effective in the treatment of CD (Bourne et al. 2008).

Crohn's Disease: Three anti-TNF agents infliximab, adalimumab, and certolizumab have been shown to be effective for induction and maintenance therapy in Crohn's disease.

Infliximab: Infliximab is a chimeric IgG1 anti-TNF antibody administered by intravenous infusion. Infliximab is effective as induction therapy for the treatment of moderate to severe Crohn's disease (Targan et al. 1997). Studies have demonstrated that scheduled maintenance therapy every 8 weeks is associated with increased remission rates, reduced steroid utilization, reduced development of antibodies of infliximab, and fewer hospitalizations (Rutgeerts et al. 1999; Hanauer et al. 2002; Rutgeerts et al. 2004). Infliximab has also been shown to be effective in reducing drainage and closing perianal and abdominal fistulas (Present et al. 1999).

Adalimumab is a fully humanized IgG1 anti-TNF antibody. Certolizumab pegol is an anti-TNF F(ab)' fragment linked to polyethylene glycol. Adalimumab and certolizumab are administered subcutaneously and are effective as induction and maintenance therapy for moderate to severe CD. Although

Adalimumab, certolizumab, and infliximab have not been directly compared, induction and remission rates are similar based on data from RCT (Hanauer et al. 2006; Rutgeerts et al. 2006; Sandborn et al. 2007; Schrieber et al. 2007). Like infliximab, adalimumab and certolizumab appear to be effective at inducing and maintaining fistula closure (Colombel et al., 2007; Screiber et al. 2011). Adalimumab and certolizumab are effective in patients who have lost response to or are intolerant of infliximab, although response rates are reduced compared to anti-TNF-naïve patients (Schreiber et al. 2007; Sandborn et al. 2007, 2010; Colombel et al. 2007; Feagan et al. 2011).

Ulcerative Colitis: Infliximab has been found to be effective for the induction of remission and maintenance in moderate to severe ulcerative colitis. Patients receiving infliximab have reduced steroid requirements and healing of the colonic mucosa (Rutgeerts et al. 2005). Recent data has also suggested that infliximab may be used as rescue therapy in severe, IV steroid-refractory ulcerative colitis (Janerot et al. 2005).

A recent small study of adalimumab in patients with moderate to severe UC has suggested efficacy in induction of remission (Sandborn et al. 2010). Studies assessing the effectiveness of additional anti-TNF agents approved for Crohn's disease (adalimumab and certolizumab) are ongoing.

Natalizumab

Mechanism of Action: Natalizumab is a humanized monoclonal antibody that targets the alpha4 integrin. Alpha4 in a complex with beta7 integrin is expressed on lymphocytes homing to the gastrointestinal tract, where its ligand, mucosal addressin cell adhesion molecule-1 (MAdCAM-1), is expressed on high endothelial venules (HEVs) within the lamina propria. Natalizumab prevents binding of alpha4 with MAdCAM-1 and limits entry of lymphocytes into gastrointestinal tissues.

Crohn's Disease: In a trial where inclusion criteria included elevated CRP, a significant increase in response and remission was achieved in patients with Crohn's disease receiving natalizumab (Targan et al. 2006). Maintenance trials have yielded consistently positive results in patients who responded to natalizumab (Sandborn et al. 2005; Panaccione et al. 2006).

Ulcerative Colitis: No randomized controlled trials of natalizumab in UC have been published to date.

Patients receiving natalizumab are at risk for developing progressive multifocal leukoencephalopathy (PML), a debilitating neurologic disease that is frequently fatal. PML occurs from reactivation of JC virus, and the risk of development is 1/1000 (Sandborn et al. 2006).

Special Considerations

Combination Therapy of Immunomodulators and Anti-TNF Therapy for Moderate to Severe CD

A recent study of patients naïve to therapy with moderate to severe CD has suggested that combination therapy with an immunomodulator plus an anti-TNF agent resulted in increased remission rates at 1 year compared to monotherapy with either drug alone (Colombel et al. 2010). However, there has been some concern regarding the safety of combination therapy, particularly with regard to a rare lymphoma called hepatosplenic T cell lymphoma (HSTCL). HSTCL is a rare, usually fatal, hematologic malignancy that has been associated with immunosuppressive use in young males (Kotlyar et al. 2011). Thus, the risks of serious complications must be weighed against the benefit of therapy on an individual basis.

Postoperative Prophylaxis in CD

Following surgical resection, endoscopic recurrence occurs in 28–93 % of patients at 1 year (Achkar et al. 2000), and clinical recurrence rates are 20 % and 34 % at 1 and 3 years (Rutgeerts et al. 1990). One-third of patients will ultimately require repeat surgery within 10 years (Achkar et al. 2000). Identifying strategies that prevent postoperative recurrence is of great interest.

Results from two meta-analyses have demonstrated that postoperative azathioprine or 6MP was associated with a significantly decreased risk of clinical recurrence and endoscopic

recurrence of disease. Studies of mesalamine have shown minimal prevention of relapse and have been shown to be inferior to 6MP in the postoperative setting (Camma et al. 1997; Lochs et al. 2000; Sutherland et al. 2000; Hanauer et al. 2004). Postoperative therapy with metronidazole (20 mg/kg/day) for 3 months resulted in fewer endoscopic lesions at 1 year and delayed clinical recurrence (Rutgeerts et al. 1995). Ornidazole 1 g/day given for 1 year and started immediately after resection also reduced endoscopic recurrence at 1 year (Rutgeerts et al. 2005). Clinical use of these antibiotics has generally been limited by peripheral neuropathy and gastrointestinal side effects. A small randomized controlled study of infliximab for postoperative prophylaxis showed significant benefit over placebo in rates of endoscopic recurrence (Regueiro et al. 2009). Further evaluation of a larger cohort of patients is necessary to determine the role of anti-TNF therapy in the postoperative setting.

Surgical Management in IBD

Surgery for Ulcerative Colitis

Despite medical options for therapy, 8–30 % of patients with UC will ultimately require colectomy (Ananthakrishnan et al. 2009). Elective surgery is indicated for medically refractory disease in patients with ongoing moderate symptoms or steroid dependency despite maximal medical therapy. In addition, elective surgery is indicated for the treatment of colitis-associated cancer or dysplasia. Options for elective surgical management in UC include total proctocolectomy with end ileostomy or restorative proctocolectomy, most commonly with ileoanal pouch anastomosis (IPAA). Total proctocolectomy with end ileostomy may be recommended for patients with impaired anal sphincter function, rectal cancer, or those unwilling or unable to undergo a multistage procedure. Complications of this procedure may include stomal stenosis, parastomal hernia, and skin breakdown, occasionally requiring stomal revision. Elective IPAA is commonly performed in two or three stages and involves the generation of a neo-rectum from the ileum anastomosed to the anal transition zone. A diverting ileostomy is performed at the time of pouch creation to allow the anastomosis to heal prior to restoration of continuity. Complications of IPAA include anastomotic separation, pouch fistulas, and pouch inflammation, called "pouchitis." A small percentage of patients (less than 5 %) have severe pouch complications requiring pouch excision (Stahlber et al. 1996; Simchuk et al. 2000). IPAA is associated with significantly reduced fertility in women (Waljee et al. 2006). In addition, patients with IPAA have an average of six loose bowel movements per day including nocturnal bowel movements. IPAA can be performed with a hand-sewn or stapled anastomosis, the latter being required when laparoscopic surgery is performed. Stapled anastomosis leaves a small rectal cuff that is at risk for development of inflammation called "cuffitis" and also for development of precancerous changes. Despite these potential complications, health-related quality of life is significantly improved in patients undergoing IPAA surgery (Heikins et al. 2010). The indications for urgent colectomy in UC include acute severe colitis not responding to medical therapy, toxic megacolon, or perforation. In the emergent setting, subtotal abdominal colectomy with ileostomy is performed. Following a period of recovery, elective IPAA or a completion proctectomy may be performed.

Surgery for Crohn's Disease

Epidemiologic data collected prior to the common use of anti-TNF agents has demonstrated that after 10 years of disease, 30–50 % of patients with CD require surgery. While new data suggest that anti-TNF therapies are having a modest impact on surgical rates, surgical intervention remains an important therapy for CD. In addition, postoperative recurrence rates are about 50 % at 10 years and many of these patients will eventually require a second operation (Bernstein et al. 2012). The major indications for the surgical management of Crohn's disease include disease refractory to medical therapy, management of

stricturing or penetrating complications, malignancy or dysplasia, and fecal diversion for severe perianal CD.

The appropriate surgery for Crohn's disease is based on disease behavior and location. In treating small intestinal disease, conservation of bowel is important given the high rate of recurrence and reoperation. Studies have suggested that large disease-free resection margins are not necessary and that gross inspection of bowel is adequate in determining length of resection rather than histology (Fazio et al. 1996). In addition, when multiple or long segments of strictures are present, stricturoplasty may be performed to conserve small bowel length. Surgical therapy may also be indicated for mesenteric abscesses, enterocutaneous, enterovesicular, or enteroenteric fistulas. For segmental Crohn's colitis, a meta-analysis of segmental resection versus total abdominal colectomy with ileorectal anastomosis has suggested similar outcomes, although there is a longer time to recurrence in patients following total colectomy (Tekkis et al. 2006). In the case of extensive colitis involving the rectum, IPAA results in a high rate of morbidity in CD including pouchitis, fistula, and pouch failure. It is therefore recommended that these patients undergo total proctocolectomy with end ileostomy. Surgery may be indicated in patients with perianal abscess or fistula. For simple abscesses, incision and drainage procedures may be adequate. In the case of fistulas, exam under anesthesia with seton placement, plug, or mucosal advancement flaps may be performed. For severe medically and surgically refractory perianal disease, diverting ileostomy may be temporarily effective in 80 % of patients. However, restoration of continuity is rarely successful (Yamomoto et al. 2000).

Cancer and IBD

Colorectal cancer has been recognized as a consequence of longstanding inflammatory bowel disease for almost 100 years (Crohn et al. 1925). A meta-analysis in 2001 including over 100 studies suggested that the risk of development of colon cancer was 2 % by 10 years, 8 % by 20 years, and 18 % by 30 years. Recent data also suggest that patients with extensive Crohn's colitis share similar increased risk.

There are distinct biologic differences between sporadic colon cancers and colitis-associated cancers (CAC). In sporadic colon cancer, carcinoma typically develops from a raised localized dysplastic precursor lesion, the adenomatous polyp. However, in the setting of IBD, dysplasia can be flat or polypoid and is often multifocal, suggesting the presence of a common underlying defect.

The molecular events leading to dysplasia also have some distinct differences. In sporadic tumors, loss of function of APC gene is an early event that leads to formation of a sporadic adenoma. Loss of p53 function occurs late and is important for the adenoma to carcinoma sequence. While chromosomal instability occurs with the same frequency in CAC, APC loss of function occurs less frequently and is a late event, while p53 mutations occur early and can be present in adjacent non-dysplastic tissue.

Several lines of evidence suggest that chronic inflammation is a key factor in predisposing to dysplasia. The risk of colon cancer in patients with IBD increases with duration and extent of disease. Further, a greater histologic severity of IBD is associated with an increased risk of colorectal cancer (Rutter et al. 2004; Gupta et al. 2007). Other risk factor for development of CAC is a family history of colorectal cancer which imparts a 2-fold increased risk. In addition, Primary Sclerosing Cholangitis (PSC) appears to be an independent risk factor for development of colitis-associated colon cancer reported in some, but not all studies.

Endoscopic surveillance colonoscopy has been shown to reduce the development of colorectal cancer in IBD (Itzkowitz et al. 2004) and to detect colon cancers at an earlier stage (Collins et al. 2006). Current surveillance

recommendations are that colonoscopy should commence at 7–8 years after diagnosis of ulcerative colitis or extensive Crohn's colitis and consist of four quadrant nontargeted biopsies every 10 cm with biopsies taken of any raised lesions or strictures. Surveillance should be performed every 1–2 years. PSC patients undergo a screening colonoscopy at the time of diagnosis to determine whether colitis is present. In patients with PSC and colitis, it is recommended that screening for dysplasia begins at the time of diagnosis. Patients with low-grade or high-grade dysplasia identified in flat tissue should be considered for colectomy. Newer techniques for surveillance colonoscopy, such as chromoendoscopy, have been shown to improve the diagnostic yield of surveillance, but are not standard of care.

Conclusions

The inflammatory bowel diseases, Crohn's disease and ulcerative colitis, are chronic conditions that result from inappropriate inflammation in the intestinal tract. Clinical presentation is characterized by diarrhea with or without blood, abdominal pain, weight loss, and systemic inflammation. The diagnosis is confirmed by pathologic examination demonstrating acute and chronic intestinal inflammation. IBD is thought to result from an inappropriate immune activation in response to the commensal bacterial flora. Medical therapies for IBD are generally aimed at suppressing the adaptive arm of the immune response. However, surgery remains an important adjunct to medical therapy for both Crohn's disease and ulcerative colitis.

Cross-References

- ▶ Erythema Nodosum
- ▶ Normal immune function and barrier: epithelial barrier
- ▶ Primary Sclerosing Cholangitis: Clinical and Systemic Manifestations and Treatment
- ▶ PTPN22
- ▶ Spondyloarthritis: Ankylosing Spondylitis
- ▶ TGF-β
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the liver

References

Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology. 2011;140:1704–12.

Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 2011;140:1785–94.

Hwang JM, Varma MG. Surgery for inflammatory bowel disease. World J Gastroenterol. 2008;14(17):2678–90.

Kaser A, Blumberg RS. Autophagy, microbial sensing, endoplasmic reticulum stress, and epithelial function in inflammatory bowel disease. Gastroenterology. 2011;140:1738–47.

Kornbluth AA, Sachar DB. Ulcerative colitis practice guidelines in adults: American college of gastroenterology, practice parameters committee. Am J Gastroenterol. 2010;105:501–23.

Kozuch PL, Hanauer S. Treatment of inflammatory bowel disease: a review of medical therapy. World J Gastroenterol. 2008;14(3):352–77.

Larsen S, Bendtzen K, Nielsen OH. Extraintestinal manifestations of inflammatory bowel disease: epidemiology, diagnosis, and management. Ann Med. 2010;42:97–114.

MacDonald TT, Monteleone I, Fantini MC, Monteleone G. Regulation of homeostasis and inflammation in the intestine. Gastroenterology. 2011;140:1768–75.

Osterman MT, Lichtenstein GR. Ulcerative colitis. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's gastrointestinal and liver disease, vol. 9. Philadelphia: Saunders; 2010. p. 1975–2012.

Rabinowitz KM, Wang Y, Chen EY, Hovhannisyan Z, Chiang D, Berin MC, Dahan S, Chaussabel D, Ma'ayan A, Mayer L. Transforming growth factor β signaling controls activities of human intestinal CD8(+)T suppressor cells. Gastroenterology. 2013;144(3):601–612.

Sands BE, Siegel CA. Crohn's disease. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's gastrointestinal and liver disease, vol. 9. Philadelphia: Saunders; 2010. p. 1941–75.

Ullman TA, Itzkowtiz SH. Intestinal inflammation and cancer. Gastroenterology. 2011;140:1807–16.

Innate Immune Cells in the Liver

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Synonyms

Acute-phase response; Cytokines; Defense; Liver regeneration; Lymphoid lineage; Myeloid lineage; Progenitors

Definition

Large populations of innate immune cells reside in healthy liver, poised to detect potential harm, damage, or infection, locally or systemically. The factors produced by these cells are critical to successful initiation and resolution of inflammation. Over- or underproduction of these immunomodulatory and effector proteins will lead to pathology.

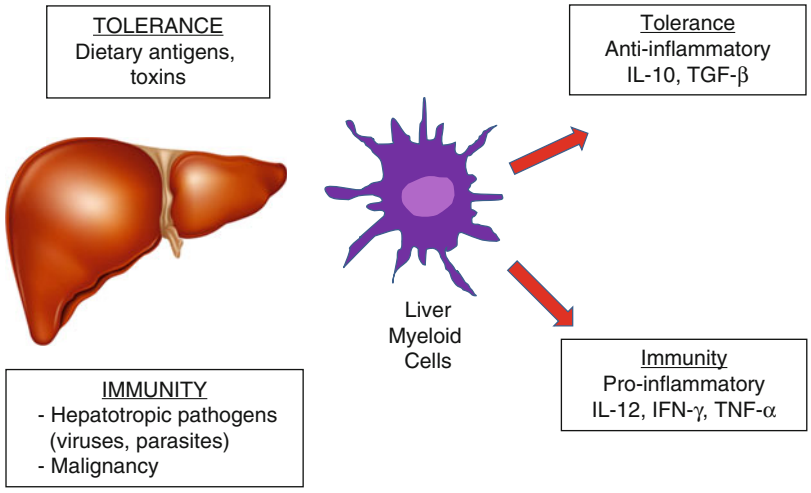
Introduction

The liver is a site of complex immune activity, capable of responding to major pathological challenges such as metastasis and infection, while remaining tolerant of most of the foreign material it encounters (O'Farrelly and Crispe 1999; Doherty and O'Farrelly 2000; Racanelli and Rehermann 2006). In its healthy state, the liver immune system tolerizes harmless proteins of dietary and commensal origin that are constantly being transported from the gut by the portal system. However, the oxygenated, nutrient-rich environment of the healthy liver provides a natural target for all types of pathogens including viruses, bacteria, fungi, nematodes, and protozoa, while constant perfusion with circulating blood makes the liver a primary target for metastasis. A complex repertoire of immunocompetent cells in the liver, in particular large populations of innate immune cells, including macrophages, dendritic cells (DCs), and natural killer receptor + (NKR+) lymphoid cells, makes sure that pathogenic and metastatic challenges are identified and dealt with appropriately, while harmless molecules and cells are tolerated (O'Farrelly and Crispe 1999; Gao et al. 2008, Fig. 1). Hepatocytes and endothelial cells also contribute to local and systemic inflammation and should be considered members of the hepatic innate cellular catalogue. The anatomical location of the liver and its rich blood supply, together with its complex collection of immune surveillant cells, equip it for detection of any signs of danger or damage in other parts of the body. Molecular signals, carried to the liver from sites of inflammation, infection, or damage, are detected by hepatocytes, which are activated to synthesize acute-phase proteins. These alert the whole body to danger, mobilizing immune components, opsonizing pathogens, and inducing cellular proliferation and additional synthesis of cellular and molecular immune components. So, the products of liver cells initiate, mediate, regulate, and resolve systemic and local immune responses and also contribute to the liver pathology often associated with inflammatory and immunological activity (Liaskou et al. 2012). Reptilian, avian, and amphibian livers also have roles in local and systemic defense, as does the "fat body," the organ responsible for metabolism in insects. Co-localization and coevolution, in such disparate species, of immunological and metabolic activities, requiring effective circulation for efficient implementation, emphasizes a highly conserved role for the liver in mediating the interphase between metabolism and defense. Key to this role are the innate immune cell populations of myeloid and lymphoid lineages that reside in healthy liver (Doherty and O'Farrelly 2000; Gao et al. 2008).

Hepatic Innate immune Cells: Myeloid Lineage

Liver Macrophages (Kupffer Cells)

Myeloid cell populations in the liver are particularly varied, with considerable overlap among

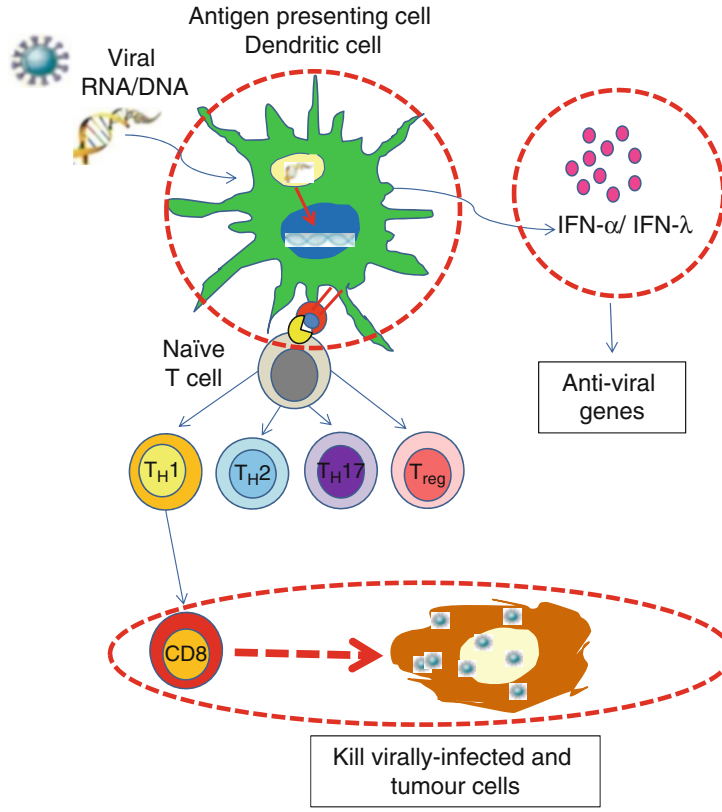


Innate Immune Cells in the Liver, Fig. 1 Liver myeloid cells contribute to both tolerance and immunity in the liver by secreting cytokines

fixed and newly resident macrophages, dendritic cells, myeloid progenitors, and myeloid regulatory cells (Lloyd and Phillips 2008). Liver-resident macrophages (also known as Kupffer cells), the primary innate immune cell type in the liver, are equipped with powerful pathogen detection and protein production potential (McCuskey et al. 1987; Bilzer et al. 2006). Kupffer cells represent 80–90 % of all tissue-fixed macrophages of the body, and 15 % of the total cell population in the healthy liver, where they are interspersed with fenestrated liver sinusoidal endothelial cells. Kupffer cells are therefore the most numerous components of the "reticuloendothelial system," characterized by versatile phagocytic function, found at sites of potential pathogenic invasion including the gut, skin, and lungs, as well as liver. Macrophages recognize microbes and their products through pattern recognition receptors (PRRs) that stimulate cell activation and pathogen phagocytosis (Medzhitov and Janeway 2002). Major protein synthesis is induced, resulting in production of antimicrobial peptides, chemokines, and cytokines, especially TNF, IL-1, IFNγ, and IL-6. Liver macrophages are potent producers of inflammatory cytokines and therefore they can help induce, mediate, and regulate systemic as well as local inflammation (O'Farrelly and Crispe 1999; Gao et al. 2008; Sitia and et al. 2011; Nemeth et al. 2009). In the liver, these messenger molecules stimulate hepatocyte synthesis of acute-phase proteins which are critical for driving, mediating, and regulating the systemic response to inflammation and ultimately restoring homeostasis (Moshage 1997; Baumann and Gauldie 1994; Gabay and Kushner 1999; Bode et al. 2012). Liver-derived acute-phase proteins, complement components, and inflammatory cytokines have major impact on liver repair and regenerative capacity (Diehl 2000; Strey et al. 2003; Rutkowski et al. 2010) and are known to influence inflammatory, physiological, and homeostatic activities in other organs, such as the brain, bone marrow, and hypothalamic-pituitary axis. In addition to their role in local and systemic inflammation, subpopulations of liver macrophages have significant regulatory activity and may contribute to the tolerogenic potential of the liver (Callery et al. 1989).

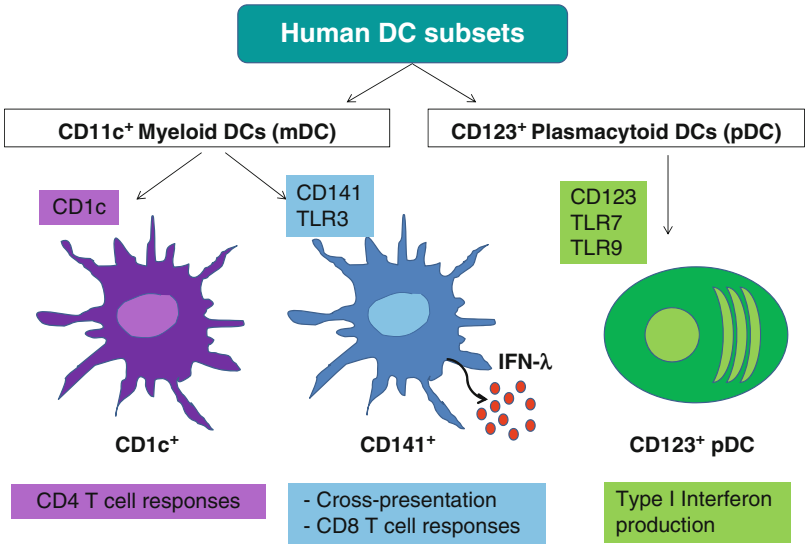
Dendritic Cells

Dendritic cells (DCs), also powerful phagocytic cells of the myeloid lineage and potent producers of messenger molecules, such as cytokines, chemokines, and growth factors (Steinman 1991), are found in much smaller numbers in healthy liver (Thomson and Knolle 2010). Classically, DC play an important role as antigen-presenting cell in initiating and directing activation and polarization of T lymphocytes in lymph nodes (Shortman and Liu 2002, Fig. 2).

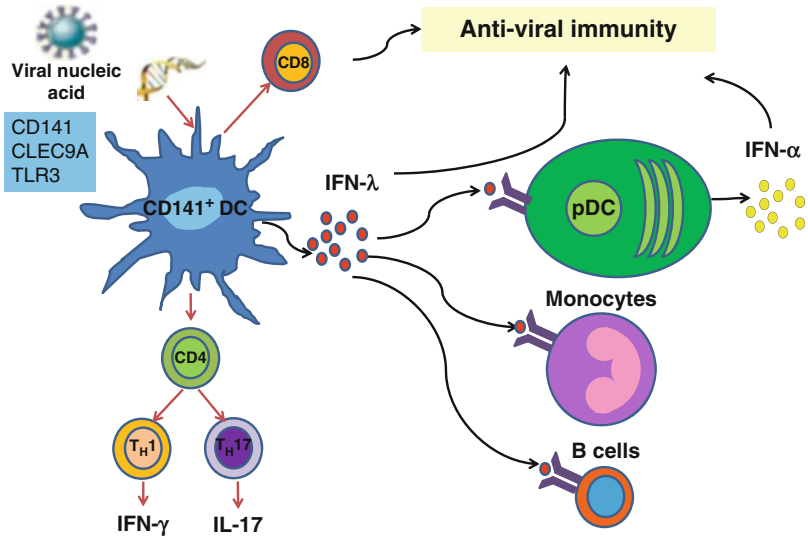


Innate Immune Cells in the Liver, Fig. 2 General role of dendritic cells as antigen-presenting cells in antiviral and antitumoral immunity

Three subsets of human DCs are classically distinguished: CD11c DCs (classical Dcs or cDCs) include CD1c+ and CD141+ subsets that activate CD4 and CD8 T cells and plasmacytoid CD123+ DCs that produce type I interferon (Fig. 2). cDCs are equipped with an extensive array of PRRs and other detection molecules such as the C-type lectin, DC-SIGN (DC-specific ICAM-3 grabbing non-integrin), and CLEC-9A and can sense the presence of pathogens, cell damage, and metastatic danger (Thomson and Knolle 2010; Doherty and O'Farrelly 2001, Fig. 3). They are major antigen-presenting cells and potent cytokine secretors, although not primarily of IFN-α (Hsu et al. 2007, Fig. 4). Plasmacytoid DCs (pDC), also well equipped with viral detection systems, are involved in antiviral responses, primarily secrete interferon-α, and are poor antigen presenters (Fig. 4). The liver contains all three DC subsets, but CD141+ DCs are more represented than in the blood (Fig. 5). Hepatic DCs often are more potent phagocytic cells and produce more cytokines than their counterparts in classical lymphoid organs, including spleen and lymph nodes (Jomantaite et al. 2004). Therefore, although present in small proportions, hepatic DCs are powerful initiators, drivers, and regulators of local innate and adaptive innate immune responses (Thomson and Knolle 2010; Doherty and O'Farrelly 2001; Jomantaite et al. 2004). Some populations of liver DCs have an immature, tolerogenic phenotype, express lower co-stimulatory molecules, secrete low levels of IL-12 and high levels of the suppressive cytokine IL-10, and thus induce anergy in naïve, allogeneic T cells (Sallusto and Lanzavecchia 1994). Subpopulations of hepatic DCs are therefore considered key drivers of immunological tolerance in the liver (Thomson and Knolle 2010; Thomson and Lu 1999; Goddard et al. 2004; De Creus et al. 2005). However, other hepatic DC populations seem capable of activating



Innate Immune Cells in the Liver, Fig. 3 Different subsets of human DCs and their role in immune responses

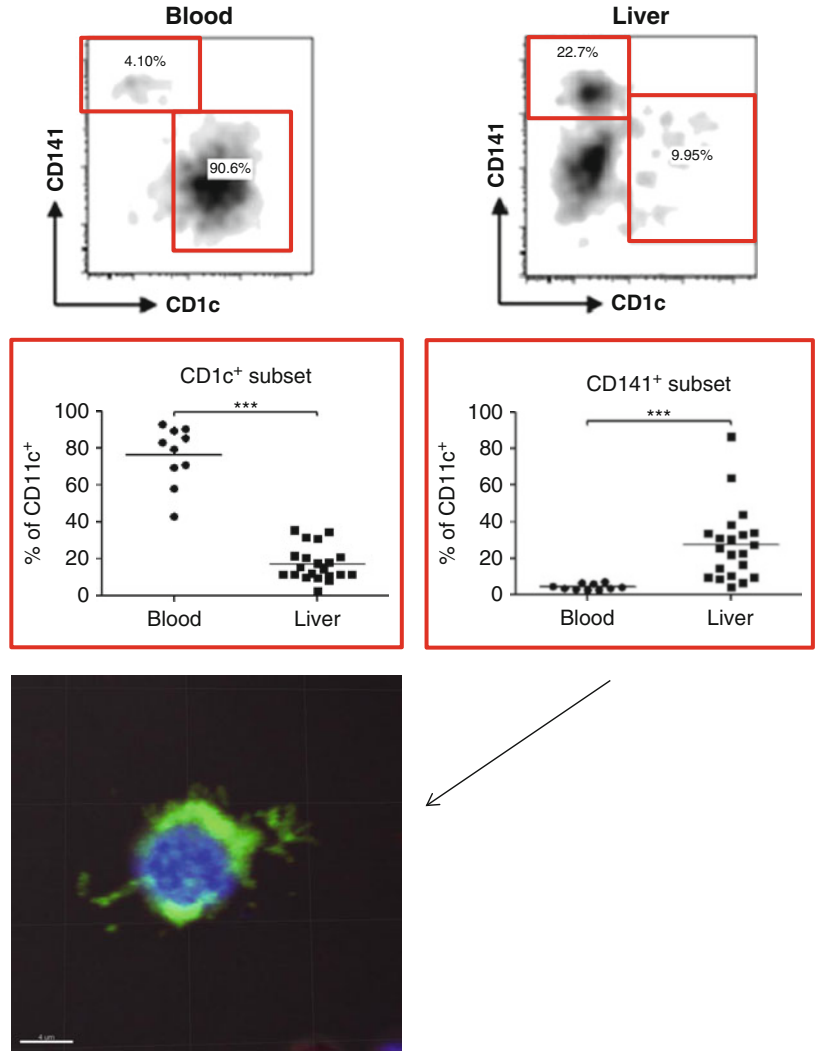


Innate Immune Cells in the Liver, Fig. 4 Role of CD141$^+$ DCs and IFN-λ in the antiviral immune response

T lymphocytes (Kelly et al. 2014) and may therefore initiate and mediate specific adaptive immunity to hepatotropic pathogens. Because hepatic DCs have such major roles in activating and regulating responses to infectious and autoimmune liver disease (Protzer et al. 2012) as well as mediating oral and portal tolerance, they have been proposed to be key to determining the balance between liver tolerance and immunity (Thomson and Knolle 2010; Doherty and O'Farrelly 2001; Duffield 2012).

Regulatory Myeloid Cells

Other heterogeneous populations of myeloid cells in the liver have potent regulatory function. Some combine features of myeloid progenitors and neutrophils and suppress T cell activity by secreting the regulatory cytokines IL-10 and TGF-β. They also produce the metabolic inhibitors, arginase and IDO, which control immune cells by depleting the immediate environment of the key amino acids, arginine and tryptophan, and are known as myeloid-derived suppressor cells



Innate Immune Cells in the Liver,
Fig. 5 Characterization of dendritic cell (*DC*) subsets in healthy donor liver perfusate

(Gabrilovich and Nagaraj 2009). These cells are considered important for inhibiting inappropriate responses to harmless antigens of dietary and commensal origin, thus promoting the tolerogenic capacity of the liver. However, increased numbers of this population have been demonstrated in tumor-bearing liver where they seem to inhibit antitumor immunological activity and so facilitate tumor growth (Ilkovitch and Lopez 2009). Immunoregulatory properties have also been ascribed to granulocyte populations (neutrophils, basophils, and eosinophils) often involved in the pathologies associated with inflammation and the immune response to parasites but found in tiny numbers in healthy liver (Siracusa et al. 2011; Jaeschke et al. 1996).

Hepatic Innate Immune Cells: Lymphoid Lineage

Natural Killer Receptor+Cells

The healthy liver is packed with large, heterogeneous populations of innate cells of lymphoid origin that are critical for local defense against viruses and tumors as well as homeostasis (Doherty and O'Farrelly 2000; Crispe 2011). These innate lymphoid cells, originally termed

"pit cells" (Hata et al. 1991), are characterized by expression of natural killer receptors (NKR) and include classical natural killer cells and populations of T lymphocytes which combine phenotypic and functional characteristics of T cells and natural killer cells (Wisse et al. 1976; Norris et al. 1998; Crispe and Mehal 1996). All respond swiftly to stimulation, producing large amounts of cytokines and having potent killing capabilities, and are therefore major components of the hepatic innate immune arsenal, with particular roles in tumor surveillance and antiviral immunity.

Natural Killer Cells: Antiviral, Antitumor, and Tissue Remodeling Activities

NK cells are more abundant in the human liver than any other organ (Crispe and Mehal 1996; Gao et al. 2009) accounting for 30–50 % of intrahepatic lymphoid cells. Hepatic NK cells have critical roles in the immune response against hepatotropic viruses such as hepatitis C and are also important components of the hepatic antitumor immune repertoire. NK cells detect changes in membrane glycoprotein expression on target cells, usually induced by viral infection, proliferation, or transformation – in other words, they detect "altered self" (Biron et al. 1999; Caligiuri 2008). When activated, NK cells kill their targets by delivering cytotoxic granules into bound target cells. NK cells are also potent cytokine producers, with subpopulations secreting so much cytokine protein that they are thought responsible for the liver pathology that often accompanies the response to local infection or malignancy (Caligiuri 2008). We now know that NK cells also have potent tissue regeneration and remodeling capabilities (Vivier et al. 2009) and must have equivalent roles in the liver. Differential expression of NK cell surface molecules, cytokine receptors, and cytoplasmic cytokines identifies subpopulations of NK cells in the liver that are responsible for local responses to pathological challenge, resulting in pathogen clearance, tissue remodeling, and return to homeostatic normality or liver scarring and damage.

The hepatic cytokine milieu, generated by local myeloid and lymphoid populations, critically influences NK phenotype and activity. In particular, G-CSF, GM-CSF, IFN-α, IFNγ, IFNλ, IL-2, IL-12, and IL-15 all impact on differentiation, phenotype, and function of NK cells and other hepatic immune cells. DC populations are potent producers of these cytokines (Shortman and Liu 2002), emphasizing the complex interplay between these two innate immune populations required for effective homeostasis or immune activity in either healthy or challenged liver.

Innate (Natural) T Lymphocytes

Classical T cells express variant receptors that are coded for by gene segments which undergo rearrangement during T cell development in the thymus. This process results in a myriad of TCR specificities (in the region of 10^9). Because of positive and negative selection in the thymus, successfully matured T cells have TCRs that only recognize foreign antigen presented in the context of self class 1 or class II major histocompatibility (MHC) molecules. In the periphery, activation of these T cells requires appropriate ligand recognition, co-stimulation, and cytokine environment. Populations of T cells have been discovered that express TCRs of limited specificity, that are coded for by restricted classes of gene segments, and that are not restricted by classical class 1 or class II major MHC molecules. Because of limited gene usage and lack of extra nucleotide insertion during the gene rearrangement events that accompany T lymphocyte development, these T cells are termed "invariant" (Bendelac et al. 2007).

One population of invariant NKT cells (iNKT) with potent antitumor capability (Bendelac et al. 2007; Cui et al. 1997) is found in relatively large numbers in human liver (Norris et al.). These iNKT cells recognize glycolipids presented by the nonclassical MHC molecule CD1d (Bendelac et al. 2007). Other invariant T cell populations include mucosal-associated invariant T (MAIT) cells which are CD161+, IL-17-secreting cells with antimicrobial activity and are restricted by the stress molecule MR1. These cells are found in high numbers in the gastrointestinal tract (Dusseaux et al. 2011) and in the liver (Billerbeck et al. 2010).

Other invariant T cell populations, in particular γδ TCR+ T cells, which recognize conserved molecules on cell surfaces such as MICA and MICB, often induced by cellular stress caused by infection or transformation are found in higher numbers in the liver than in the circulation (Crispe 2011; Norris et al.). One subpopulation of γδ TCR+ T cells (expressing Vδ3) seems to be specific to the liver (Kenna et al.). Expression of CD161 by the majority of these populations is an indication of their targeted homing (Norris et al.; Billerbeck et al. 2010; Lalor and Adams 2002).

Innate Immune Populations May Differentiate and Mature in Adult Liver

Major advances are being made in identifying and defining how populations of immune cells traffic to the liver during health and disease (Lalor and Adams 2002; Lee and Kubes 2008; Shetty et al. 2008). However it remains possible that some of these cells differentiate locally (Sato et al. 1993; Golden-Mason and O'Farrelly 2002). The myelopoietic and lymphopoietic potential of fetal liver is well recognized (Payushina 2012). Healthy adult liver also seems to support self-renewal of hepatic macrophage and innate lymphoid cell populations as well as differentiation from liver-derived, hematopoietic stem cells (HSCs). Functional HSCs have been demonstrated in mouse (Li et al. 2006), rat (Yovchev et al. 2008), and human adult liver (Crosbie et al. 1999; Golden-Mason et al. 2000) capable of proliferation and differentiation. Moreover, high levels of IL-7 and IL-15, two cytokines required for lymphoid maturation and activation, are found in healthy liver (Golden-Mason et al. 2001, 2004). Significant proportions of hepatic HSCs express lymphoid and myeloid lineage markers as well as the receptors for IL-7 and IL-15, suggesting that they are progenitors of hepatic macrophage and innate lymphoid populations. HSCs expressing the myeloid marker CD33 are expanded in tumor-bearing liver (Golden-Mason et al. 2000) and are thought to be responsible for increased immature, neutrophil populations with suppressor function seen in tumor-bearing liver tissue.

Nonclassical Innate Immune Cells in the Liver (Hepatocytes, Epithelial, and Endothelial Cells) and the Acute-Phase Response

Classically, innate immune cell populations have included myeloid phagocytic cells and NKR+ lymphoid cells exclusively. However, other nonimmune cell types, particularly in the liver, have key roles in recruiting and homing of immune cells (Edwards et al. 2005) as well as in local and systemic innate immune responses; they should therefore be considered components of the hepatic innate immune system. The acute hepatic response to systemic injury requires re-prioritization of liver protein synthesis by all hepatic cells, particularly hepatocytes as well as endothelial and epithelial cell populations. Inflammatory cytokines and other stress factors produced at distal sites of inflammation, are detected by receptors on nonhematopoietic as well as immune cells. These factors may induce a major shift of available synthetic capacity and amino acid resources from constitutive production of proteins such as albumin and transferrin, to production of non-constitutive acute-phase proteins (APP). Positive acute-phase reactants include fibrinogen, pentraxins such as C-reactive protein (CRP), serum amyloid A, and haptoglobin as well as effector molecules such as complement components, defensins, and hepcidin (Edwards et al. 2005; Rowell et al. 1997; Strnad et al. 2011). These multipotent molecules alert the body to potential danger, mediate significant antimicrobial activity, and drive appropriate systemic physiological, metabolic, and immunological responses. They therefore have major roles in the restoration of homeostasis after injury and metabolic stress as well as inflammation and defense.

Conclusion

Relative over- and underproduction of acute-phase proteins, cytokines, and growth factors in the liver, products of myeloid and lymphoid innate immune cells, determine the clearance efficiency of any inflammatory or pathological

insult locally or anywhere in the body. They will also regulate the effectiveness of local and distal inflammatory activity, act to resolve inflammation and regenerate tissue, as well as control the extent of resulting hepatic scarring. The liver innate immune system is therefore pivotal to immunological and physiological homeostasis.

Summary

The liver is in constant monitoring mode, not only of metabolic activity and the body's metabolic requirements but also of signs of potential or actual internal harm anywhere in the body. If none are detected, the main focus for the organ remains normal physiological and metabolic activity, which requires immunological homeostasis. If under pathological threat, the liver switches from metabolic mode towards mobilizing itself and the body for defense. Innate immune cells are responsible for many of the detection, regulatory, and effector activities required for immune homeostasis and responsiveness in healthy and diseased liver.

Cross-References

- Acute and Chronic Hepatitis B Virus Infection, Immune Response
- Adaptive Immune Cells in the Liver
- Animal Models of Autoimmune Hepatitis
- Animal Models of Hepatitis B and C
- Cell Adhesion Molecules
- Chemokines
- Cytotoxic T Lymphocytes
- Endothelial Cells and Inflammation
- Gestational Alloimmune Liver Disease
- Hepatic Lymphatic System
- Immune Responses to the Hepatitis C Virus
- Immunosuppression in Clinical Liver Transplantation
- Interleukin-6
- Kupffer Cells in Immune Tolerance
- Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance
- Liver Transplantation Tolerance in Animal Models for Encyclopedia of Medical Immunology
- Liver Vasculature and Microvasculature
- Macrophages, Oxidative Stress, and Atherosclerosis
- Mammalian Target of Rapamycin (mTOR)
- Neutrophils
- Neutrophils in Endothelial Damage
- NF-κB
- NK Cell Activation
- Normal Immune Function and Barrier: Defensins
- Normal Immune Function and Barrier: Epithelial Barrier
- Normal Immune Function and Barrier: Gamma Delta T-Cells
- Normal Immune Function and Barrier: Vitamin D
- Nuclear Factor of Activated T Cells (NFAT)
- PBC Genetics
- Primary T-Cell Activation in Liver
- Regulatory B Cells
- Resolution of Inflammation
- TGF-β
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- Tregs in the Liver
- Tumor Macrophages
- Tumor-Infiltrating T Cells
- Ultrastructure of the Liver Sinusoid

References

Baumann H, Gauldie J. The acute phase response. Immunol Today. 1994;15:74–80.

Bendelac A, Savage P, Teyton L. The biology of NKT cells. Annu Rev Immunol. 2007;25:297–336.

Billerbeck E, Kang YH, Walker L, Lockstone H, Grafmueller S, et al. Analysis of CD161 expression on CD8 T cells defines a distinct functional subset with a tissue homing properties. Proc Natl Acad Sci USA. 2010;107:3006–11.

Bilzer M, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. Liver Int. 2006;26:1175–86.

Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol. 1999;17:189–220.

Bode J, Albrecht U, Haussinger D, Schaper F. Hepatic acute phase proteins – regulation by IL-6 and IL-1 type cytokines involving STAT3 and its crosstalk with NF-kB-dependent signaling. Eur J Cell Biol. 2012;91:496–505.

Caligiuri MA. Human natural killer cells. Blood. 2008;112:461–9.

Callery MP, Kamei T, Flye MW. Kupffer cell blockade blocks induction of tolerance by the portal venous route. Transplantation. 1989;47:1092–4.

Crispe IN. The liver as a lymphoid organ. Annu Rev Immunol. 2011;27:147–63.

Crispe IN, Mehal WZ. Strange brew: T cells in the liver. Trends Immunol. 1996;17:522–5.

Crosbie O, Reynolds M, Curry M, Nolan N, Traynor O, McEntee G, Kelly J, Hegarty J, O'Farrelly C. In vitro evidence for the presence of hematopoietic stem cells in the adult human liver. Hepatology. 1999;29:1193–8. PMID 10094964.

Cui J, Shin T, Kawano T, Sato H, Kondo E, Toura I, Kaneko Y, Koseki H, Kanno M, Taniguchi M. Requirement for Va14 NKT cells in IL12 mediated rejection of tumors. Science. 1997;278:1623–6.

De Creus A, Abe M, Lau AH, Hackstein H, Raimondi G, Thomson AW. Low TLR4 expression by liver dendritic cells correlates with reduced capacity to activate allogeneic T cells in response to endotoxin. J Immunol. 2005;174:2037–45.

Diehl AM. Cytokine regulation of liver injury and repair. Immunol Rev. 2000;174:160–71.

Doherty DG, O'Farrelly C. Innate and adaptive immune cells in the human liver. Immunol Rev. 2000;174:5–20.

Doherty D, O'Farrelly C. Dendritic cells: regulators of hepatic immunity or tolerance? J Hepatol. 2001;34:156–60.

Duffield JS. Dendritic cells take on more tasks in the liver. Hepatology. 2012;55:16–9.

Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, Milder M, Le Bourhis L, Soudauis C, Treiner E, Lantz O. Human MAIT cells are xenobiotic resistant, tissue targeted CD161hi IL-17 secreting T cells. Blood. 2011;117:1205209.

Edwards S, Lalor PF, Nash GB, Rainger GE, Adams DH. Lymphocyte traffic through sinusoidal endothelial cells is regulated by hepatocytes. Hepatology. 2005;41:451–9.

Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340:448–54.

Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009;9:162–74.

Gao B, Jeong WI, et al. Liver: an organ with predominant innate immunity. Hepatology. 2008;47(2):729–36.

Gao B, Radaeva S, Park S. Liver natural killer and natural killer T cells: immunobiology and emerging roles in liver diseases. J Leukoc Biol. 2009;86: 513–28.

Goddard S, Youster J, Morgan E, Adams DH. Interleukin-10 secretion differentiates dendritic cells from human liver and skin. Am J Pathol. 2004;164:511–9.

Golden-Mason L, O'Farrelly C. Having it all? Stem cells, haematopoiesis and lymphopoiesis in adult human liver. Immunol Cell Biol. 2002;80:45–51.

Golden-Mason L, Curry M, Nolan N, Traynor O, McEntee G, Kelly J, Hegarty J, O'Farrelly C. Differential expression of lymphoid and myeloid markers on differentiating hematopoietic stem cells in normal and tumor bearing adult human liver. Hepatology. 2000;31:1251–6. PMID 10827150.

Golden-Mason L, Kelly A, Traynor O, McEntee G, Kelly J, Hegarty J, O'Farrelly C. Expression of interleukin 7 (IL-7) mRNA and protein in the normal adult human liver: implications for extrathymic T cell development. Cytokine. 2001;14:143–51. PMID 11396992.

Golden-Mason L, Kelly A, Hegarty J, O'Farrelly C. Hepatic Interleukin 15 (IL-15) expression: implications for local NK/NKT cell homeostasis and development. Clin Exp Immunol. 2004;138(1):94–101. PMID 15373910.

Hata K, Van Thiel D, Herberman RB, Whiteside T. Natural killer activity of human-derived lymphocytes in various liver diseases. Hepatology. 1991;14:495–503.

Hsu W, Shu SA, et al. The current immune function of hepatic dendritic cells. Cell Mol Immunol. 2007;4(5):321–8.

Ilkovitch D, Lopez D. The liver is a site for tumor induced myeloid derived suppressor cell accumulation and immunosuppression. Cancer Res. 2009;69:5514–21.

Jaeschke H, Smith CW, Clemens MG, Ganey PE, Roth RA. Mechanisms of inflammatory liver injury: adhesion molecules and cytotoxicity of neutrophils. Toxicol Appl Pharmacol. 1996;139:213–26.

Jomantaite IN, Dikopoulos N, Kroger A, Leithauser F, Hauser H, Schirmbeck R, Reimann J. Hepatic dendritic cell subsets in the mouse. Eur J Immunol. 2004;34:355–65.

Kelly A, Fahey R, Fletcher J, Keogh C, Carroll A, Siddachari R, Geoghegan J, Hegarty J, Ryan E, O'Farrelly C. CD141+ myeloid dendritic cells are enriched in healthy human liver. J Hepatol. 2014;60(1):135–42. doi:10.1016/j.jhep.2013.08.007. Epub 2013 Aug 19.

Kenna T, Norris S, Collins C. McEntee G, Traynor O, Nolan N, Hegarty J O'Farrelly C, Doherty D.

Lalor PF, Adams DH. The liver: a model of organ-specific lymphocyte recruitment. Expert Rev Mol Med. 2002;4:1–16.

Lee WY, Kubes P. Leukocyte adhesion in the liver: distinct adhesion paradigm from other organs. J Hepatol. 2008;48(3):504–12.

Li WL, Su J, Yao YC, Tao XR, Yan YB, Yu HY, Wang XM, Li JX, Yang YJ, Lau JT, Hu YP. Isolation and characterization of bipotent liver progenitor cells from adult mouse. Stem Cells. 2006;24:322–32.

Liaskou E, Wilson DV, Oo Y. Innate immune cells in liver inflammation. Mediators Inflamm. 2012;2012:949157. doi:10.1155/2012/949157. Epub 2012 Aug 9.
Lloyd CM, Phillips AR. Three-colour fluorescence immunohistochemistry reveals the diversity of cells staining for macrophage markers in murine spleen and liver. J Immunol Methods. 2008;334:70–81.
McCuskey RS, McCuskey PA, Urbashek R, Urbashek B. Kupffer cell function in host defence. Rev Infect Dis. 1987;9:S616.
Medzhitov R, Janeway C. Decoding the patterns of self and nonself by the innate immune system. Science. 2002;296:298–300.
Moshage H. Cytokines and the hepatic acute phase response. J Pathol. 1997;181:257–66.
Nemeth E, Baird A, O'Farrelly C. Microanatomy of the liver immune system. Semin Immunol. 2009;31:333–43.
Norris S, Collins C, Doherty DG, Smith F, McEntee G, Traynor O, Nolan N, Hegarty J, O'Farrelly C. Resident human hepatic lymphocytes are phenotypically different from circulating lymphocytes. J Hepatol. 1998;28 (1):84–90.
O'Farrelly C, Crispe IN. Prometheus through the looking glass: reflections on the hepatic immune system. Trends Immunol. 1999;20:394–8.
Payushina OV. Hematopoietic microenvironment in the fetal liver: roles of different cell populations. Int Sch Res Netw (Cell Biol). 2012. doi:10.5402/2012/979480.
Protzer U, Maini MK, Knolle PA. Living in the liver: hepatic infections. Nat Rev Immunol. 2012;12:201–13.
Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology. 2006;43:S54–62.
Rowell DL, Eckmann L, Dwinell MB, Carpenter SP, Raucy JL, Yang SK, Kagnoff MF. Human hepatocytes express an array of proinflammatory cytokines after agonist stimulation or bacterial invasion. Am J Physiol. 1997;273:322–32.
Rutkowski MJ, Sughrue ME, Kane AJ, Ahn BJ, Fang S, Parsa AT. The complement cascade as a mediator of tissue growth and regeneration. Inflamm Res. 2010;59:897–905.
Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. J Exp Med. 1994;179:1109–18.
Sato Y, Tsukada K, Liai T, Ohmoris K, Yoshida K, et al. Activation of extrathymic T cells in the liver during liver regeneration following partial hepatectomy. Immunology. 1993;78:86–91.
Shetty S, Lalor PF et al. Lymphocyte recruitment to the liver: molecular insights into the pathogenesis of liver injury and hepatitis. Toxicology. 2008;254(3):136–46. doi: 10.1016/j.tox.2008.08.003. Epub 2008 Aug 19.
Shortman K, Liu YJ. 2002. Mouse and human dendritic cell subtypes. Nat Rev Immunol. 2002;2(3):151–61.
Siracusa MC, Comeau MR, Artis D. New insights into basophil biology: initiators, regulators, and effectors of type 2 inflammation. Ann NY Acad Sci. 2011;1217:166–77.
Sitia G et al. Kupffer cells hasten resolution of liver immunopathology in mouse models of hepatitis. PLOS Pathogens. 2011;7(6):e1002061. doi:10.1371/journal.ppat.1002061. Epub 2011 Jun 2.
Steinman R. The dendritic cells system and its role in immunogenicity. Annu Rev Immunol. 1991;9:271–96.
Strey CW, Markiewski M, Mastellos D, et al. The proinflammatory mediators C3a and C5a are essential for liver regeneration. J Exp Med. 2003;198:913–23.
Strnad P et al. Hepcidin is an antibacterial, stress-inducible peptide of the biliary system. PloSOne. 2011;6(1): e16454. doi: 10.1371/journal.pone.0016454.
Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol. 2010;10:753–66.
Thomson AW, Lu L. Are dendritic cells the key to liver transplant tolerance? Immunol Today. 1999;20:27–32.
Vivier E, Spits H, Cupedo T. Interleukin-22 producing innate immune cells:new players in mucosal immunity ad tissue repair. Nat Rev Immunol. 2009;9:229–34.
Wisse E, van't Noordende JM, van der Meulen J, Daems TW. The pit cell: description of a new type of cell occurring in rat liver sinusoids and peripheral blood. Cell Tissue Res. 1976;173:423–35.
Yovchev MI, Grozdanov PN, Zhou H, Racherla H, Guha C, Dabeva MD. Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. Hepatology. 2008;47:636–47.

Interleukin-6

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Synonyms

26 kDa protein; B cell differentiation factor (BCDF); B cell stimulatory factor 2 (BSF-2); Hepatocyte-stimulating factor (HSF); Hybridoma growth factor (HGF); Interferon-β2 (IFNβ2)

Definition

Interleukin-6 (IL-6), originally identified as a B cell differentiation factor, is a typical cytokine featuring pleiotropic and redundant activity. It is an immunoregulatory cytokine providing host defense against infections and injuries, while dysregulation of persistent IL-6 synthesis causes the development of various diseases.

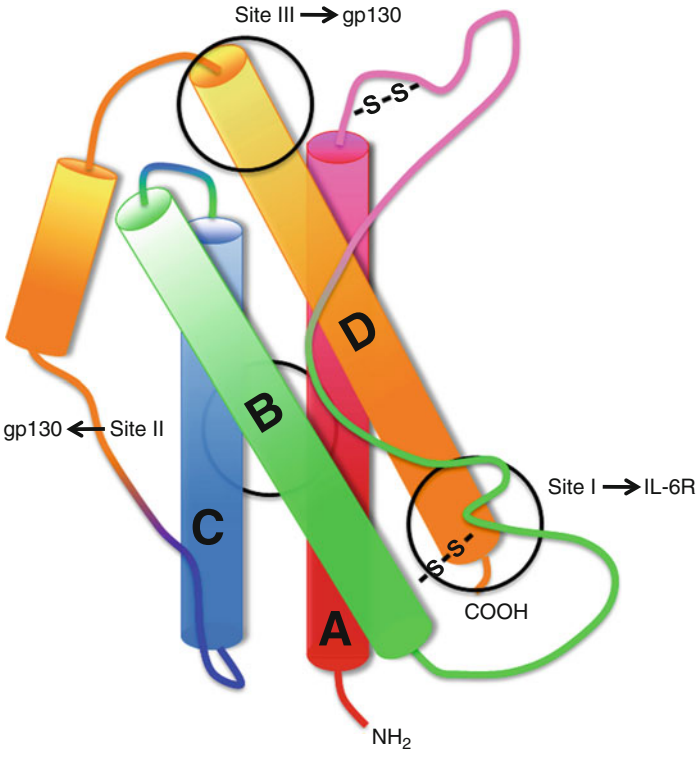
Historical Background

IL-6 was first recognized as a T cell-derived factor, which induces B cell differentiation into antibody-producing cells (Kishimoto and Ishizaka 1973). It was therefore purified and molecularly cloned on the basis of an assay for B cell differentiation activity. This cytokine was variously known as B cell-stimulating factor 2, B cell differentiation factor, or by various other names before it was recognized that all these entities were identical and the name was unified as IL-6 (Kishimoto 1989). Subsequent in vitro studies and analyses of IL-6 transgenic mice and experimental animal models have shown that IL-6 acts not only on B cells but also on T cells, hepatocytes, hematopoietic progenitor cells, and neuronal cells, in addition to performing a variety of functions (Kishimoto 1989; Akira et al. 1993). In spite of this wide range of biological activities, IL-6-deficient mice develop well except for low immune response, which indicates that IL-6 is induced by infection or injury and performs as an SOS signal (Kopf et al. 1994). Along with the identification of its multiple biological activities, numerous studies have demonstrated the pathological significance of IL-6 for various autoimmune and inflammatory diseases.

Structure of IL-6 and Its Receptor System

Human IL-6 (GenBank: X04602) consists of 212 amino acids including a 28-amino-acid signal peptide (Hirano et al. 1986), whereas mouse IL-6 (GenBank: X06203) and rat IL-6 (GenBank: M26744) consist of 211 amino acids with a 24-amino-acid signal peptide. Mature IL-6 protein contains 184 (human) or 183 (mouse) amino acids and includes two disulfide bridges between Cys44-Cys50 and Cys73-Cys83 (human) or Cys46-Cys52 and Cys75-Cys85 (mouse). The molecular mass of human IL-6 has been calculated from the amino-acid sequence as 20.7 kDa, but the weight of secreted IL-6 as 21-26 kDa, depending on posttranslational modifications such as N- or O-linked glycosylation. The human IL-6 gene has been mapped to chromosome 7p21. The structure of IL-6 includes four α-helix bundles (labelled A to D in Fig. 1) and three loops (two long A-B and C-D loops and a short B-C loop), with the four α-helix bundles arranged in an up-up-down-down topology. The amino-acid sequence of human IL-6 is 42 % homologous with that of mouse IL-6, while the homology between the sequences of mouse and rat is 93 %.

The IL-6 receptor (IL-6R) system consists of two chains, IL-6R and gp130, both of which have a Trp-Ser-X-Trp-Ser motif and belong to the cytokine receptor family. IL-6R (GenBank: X12830; alternative name: CD126) is the 80-kDa IL-6-binding subunit with an extracellular region of 339 amino acids, a membrane-spanning region of 28 amino acids and a cytoplasmic region of 82 amino acids (Yamasaki et al. 1988). The soluble form of IL-6R, which lacks the cytoplasmic region, also induces cellular responsiveness to IL-6, indicating that the IL-6 signal is transduced by another molecule. The IL-6 signal-transducing component is gp130 (GenBank: M57230; alternative name: CD130), which is a 130-kDa transmembrane glycoprotein with an extracellular region of 597 amino acids, a membrane-spanning region of 22 amino acids, and a cytoplasmic region of 277 amino acids (Hibi et al. 1990). Although IL-6R has a low IL-6 binding affinity (Kd = 5 nM), IL-6R combined with gp130 shows a high IL-6 binding affinity (Kd = 40–70 pM). The broad range of expression of gp130 on various cells explains why IL-6 has pleiotropic effects; since naturally occurring soluble IL-6R (sIL-6R) is present in human serum at



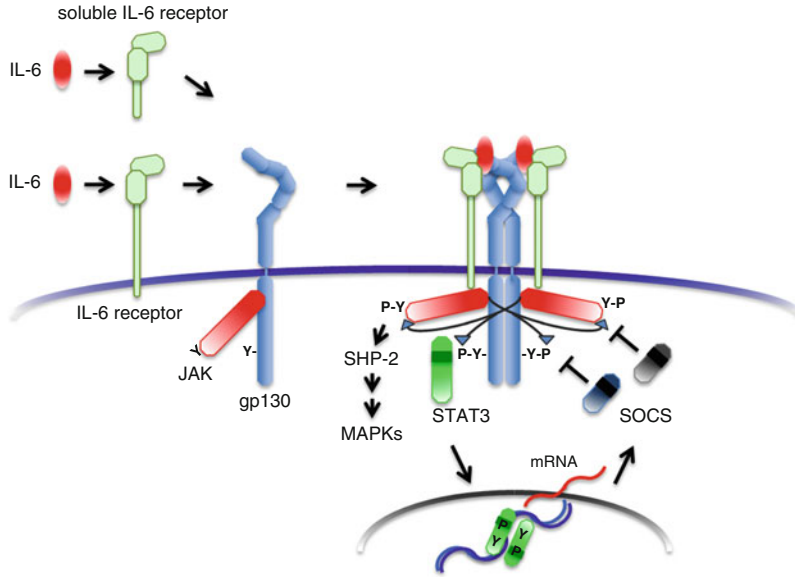
Interleukin-6, Fig. 1 Schematic representation of the domain structure of human IL-6, showing the four long α-helices, A (*red*), B (*green*), C (*blue*), and D (*orange*), and three connecting loops. Two disulfide bonds are in loop A-B. Site I on the C-terminal end of helix D interacts with IL-6R, site II located on helices A and C interacts with one gp130, and site III on the N-terminal end of helix D interacts with another gp130 (Reference; Paonessa G et al. 1995)

a concentration of around 80 ng/ml, and even in cells lacking transmembrane IL-6R, the IL-6/sIL-6R complex can transduce the IL-6 signal on gp130-expressing cells. The soluble form of gp130 is also present in human serum at a concentration of around 400 ng/ml (Narazaki et al. 1993).

After binding of IL-6 to IL-6R or soluble IL-6R (sIL-6R), the resultant IL-6/IL-6R or IL-6/sIL-6R complex associates with gp130 and the activated IL-6 receptor complex is formed as a hexameric structure comprising two molecules each of IL-6, IL-6R, and gp130. Site I of IL-6 associates with IL-6R and site II and site III interact with gp130 (Paonessa et al. 1995). Homodimerization of gp130 triggers activation of the JAK (Janus kinase)-STAT3 (signal transducer and activator of transcription 3) pathway and the JAK-SH2 domain-containing protein-tyrosine phosphatase-2 (SHP-2)-mitogen-activated protein (MAP) kinase pathway. STAT3 regulates transcription for various sets of IL-6 responsive genes including suppressor of cytokine signaling (SOCS), and then SOCS proteins turn off the IL-6 signal transduction by binding with JAK or tyrosine-phosphorylated gp130 in a negative feedback loop (Naka et al. 1997) (Fig. 2). IL-6R is a unique binding receptor for IL-6, whereas gp130 is shared by IL-6 family cytokines including leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), IL-11, cardiotrophin 1 (CTF1), cardiotrophin-like cytokine (CLC), and IL-27. These cytokines often show overlapping functions with those of IL-6. The model in which various cytokines use the common signal transducer gp130 explains clearly why various cytokines show functional redundancy (Kishimoto et al. 1992; Kishimoto et al. 1994; Kishimoto et al. 1995; Heinrich et al. 2003).

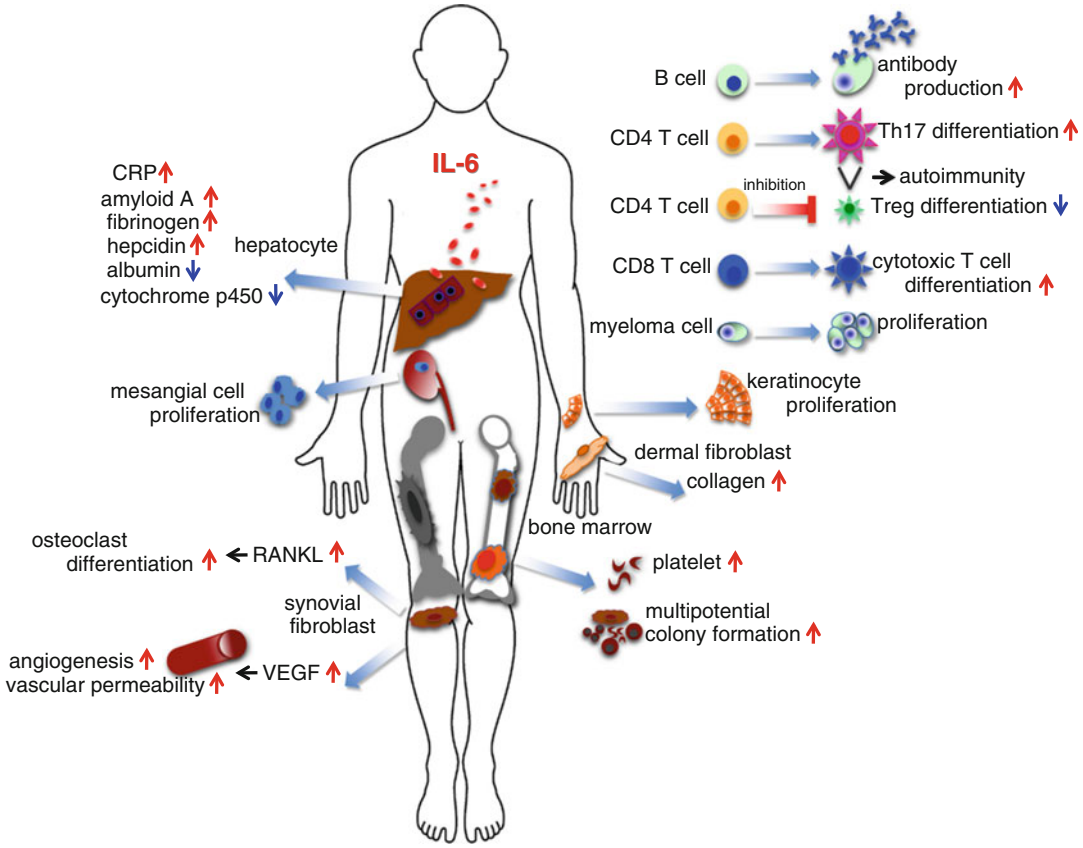
Biological Activities of IL-6

In the early phase of infectious inflammation, IL-6 is produced by monocytes and macrophages after the stimulation of Toll-like



Interleukin-6, Fig. 2 The IL-6 receptor system. After binding of IL-6 to interleukin-6 receptor (*IL-6R*), the resultant IL-6/IL-6R complex associates with gp130 and induces homodimerization of gp130, which triggers activation of JAKs and tyrosine phosphorylation of gp130. The phosphorylated gp130 then recruits STAT3 via the SH2 domain. Next, activated STAT3 translocates into the nucleus and regulates transcription for various sets of genes including SOCS. SOCS proteins bind to JAK or gp130 and turn off IL-6 signals. Tyrosine-phosphorylated gp130 also recruits SHP-2 and activates the MAP kinase pathway. Abbreviations: *JAKs* Janus kinase family tyrosine kinases, *STAT3* signal transducer and activator of transcription 3, *SOCS* suppressor of cytokine signaling, *SHP-2* SH2 domain-containing protein-tyrosine phosphatase-2, *MAP* mitogen-activated protein

receptors (TLRs) with distinct pathogen-associated molecular patterns (PAMPs). In noninfectious inflammations, such as burn or traumatic injury, damage-associated molecular patterns (DAMPs) from damaged or dying cells stimulate TLRs to produce IL-6. IL-6 possesses pleiotropic activities that play a central role in host defense (Fig. 3). IL-6 strongly induces a broad spectrum of acute-phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, hepcidin, haptoglobin, and antichymotrypsin, whereas it reduces albumin, cytochrome P (CYP) 450, fibronectin, and transferrin in liver. Changes in the levels of these proteins are also observed in acute and chronic inflammatory diseases, and prolonged inflammation leads to a pathological state. For example, high levels of hepcidin block iron transporter ferroportin 1 on macrophages, hepatocytes, and gut epithelial cells, leading to hypoferremia and anemia of chronic inflammation, whereas a high level of SAA over long periods results in amyloid A (AA) amyloidosis. In lymphocytes, IL-6 induces B cell differentiation into immunoglobulin-producing cells. When CD4-positive naïve T cells are primed, a specific cytokine directs their differentiation into an effector T cell subset. IL-6 together with TGF-β preferentially promotes differentiation of IL-17-producing T helper cells (Th17) that play a crucial role in the induction of autoimmune tissue injury, whereas IL-6 inhibits TGF-β-induced regulatory T cell (Treg) differentiation. This Th17/Treg imbalance leads to breakage of immunological tolerance and is of pathological importance for the development of autoimmune and chronic inflammatory diseases. IL-6 also acts on CD8-positive T cells to induce cytotoxic T cells. In hematopoiesis, IL-6 induces maturation of megakaryocytes into platelets as well as activation of hematopoietic stem cells.



Interleukin-6, Fig. 3 IL-6 induces cell differentiation and specific gene expression. It induces production of acute-phase proteins such as CRP, serum amyloid A, fibrinogen, and hepcidin, whereas it reduces synthesis of albumin and cytochrome P450 in hepatocytes. In addition, IL-6 promotes immunoglobulin synthesis in activated B cells and induces Th17 differentiation from naïve CD4 T cells, whereas it inhibits Treg differentiation. In bone marrow, IL-6 induces maturation of megakaryocytes into platelets and activation of hematopoietic stem cells. IL-6 also acts on synovial fibroblasts to produce RANKL and VEGF, which promote differentiation of osteoclasts and angiogenesis, respectively. Furthermore, IL-6 stimulates dermal fibroblasts to produce collagen and the growth of cells such as keratinocytes, myeloma/plasmacytoma cells, and mesangial cells. Abbreviations: *CRP* C-reactive protein, *Treg* regulatory T cells, *RANKL* receptor activator of NF-κB ligand, *VEGF* vascular endothelial growth factor

IL-6 production in bone marrow stromal cells induces the receptor activator of NF-kappaB ligand (RANKL), which is an essential factor for the differentiation and activation of osteoclasts and bone resorption, leading to osteoporosis. Enhanced angiogenesis and increased vascular permeability are pathological features of inflammation, and these characteristics are due to the excess production of vascular endothelial growth factor (VEGF), which is induced by IL-6 in inflamed lesions such as seen in synovium tissue of rheumatoid arthritis. The promotional activities of IL-6, for example, the proliferation of keratinocytes or collagen production in dermal fibroblasts, may contribute to autoimmune skin diseases including psoriasis and systemic sclerosis, respectively. Furthermore, IL-6 stimulates the growth of cells such as myeloma/plasmacytoma cells and mesangial cells.

Pathological Role of IL-6 in Development of Diseases

IL-6, when synthesized transiently, promptly participates in the host defense against

environmental stress such as infection and injury and, at the same time, provides an SOS (warning) signal by triggering a broad spectrum of biological events. Once the source of stress is removed from the host, IL-6-mediated activation of the signal transduction cascade is terminated by negatively regulated systems in conjunction with the normalization of serum IL-6 and CRP levels. However, dysregulated persistent IL-6 production has been implicated in the development of various autoimmune, chronic inflammatory diseases, and even cancers (Akira et al. 1993; Kishimoto 2005). The pathological significance of IL-6 was first demonstrated in a case of cardiac myxoma, in which excess production of IL-6 from myxoma tissues was thought to be possibly responsible for clinical symptoms and abnormal laboratory findings such as fever, arthritis, anemia, autoantibody production, and hypergammaglobulinemia (Hirano et al. 1987). Dysregulation of IL-6 production from the involved lymph nodes, synovial tissues, and myeloma cells was demonstrated to play a key role in the pathogenesis of Castleman's disease, rheumatoid arthritis, and multiple myelomas, respectively. Subsequent studies have also provided evidence of the pathological role of IL-6 in various other diseases. Elucidation of the mechanism(s) underlying persistent IL-6 synthesis in such diseases is, thus, of particular importance, while it was found that in HIV-positive cases of multicentric Castleman's diseases, all patients were infected with the Kaposi sarcoma-associated herpes virus and that sustained synthesis of both virus-derived IL-6, which directly binds to and stimulates human gp130, and host-derived human IL-6 contributes to the development of the disease.

As mentioned earlier, IL-6 is capable of breaking the immunological tolerance to autoantigens as well as of inducing systemic and local inflammation so that long-lasting overproduction of IL-6 may cause the development and progression of various autoimmune and chronic inflammatory diseases, while it also promotes growth of certain types of cancer cells, modifies immunosurveillance against cancers, and induces paraneoplastic syndrome. Numerous animal models of diseases have also shown the pathologic role of IL-6 in disease development and that IL-6 blockade by gene knockout or administration of anti-IL-6 or anti-IL-6R antibody can suppress such disease development either preventatively or therapeutically. For example, IL-6 blockade strategy demonstrated limited susceptibility to Castleman's disease-like symptoms in IL-6 transgenic mice, antigen- or collagen-induced arthritis, experimental autoimmune encephalomyelitis, experimental autoimmune uveoretinitis, spontaneous development of systemic lupus erythematosus (SLE), bleomycin-induced scleroderma, C-peptide-induced myositis, pristane-induced plasmacytomas, and other diseases.

Therapeutic Application of IL-6 Blockade Strategy for Diseases

Because of the pathological role of IL-6 in various diseases, IL-6 blockade was expected to constitute a novel treatment strategy for these diseases (Kishimoto 2005; Kishimoto 2010; Tanaka et al. 2012). Consequently, a humanized anti-human IL-6R monoclonal antibody (chemical name: tocilizumab, generic name: ACTEMRA outside of the EU or RoACTEMRA inside the EU) was developed, by grafting the complementarity-determining regions of a mouse anti-human IL-6R antibody onto human IgG1. Tocilizumab blocks IL-6-mediated signal transduction by inhibiting IL-6 binding to transmembrane and soluble IL-6 receptors. In clinical terms, if free tocilizumab concentration is maintained at more than 1 μg/ml, CRP remains negative so that serum concentration of CRP is a hallmark for determining whether IL-6 activity is completely blocked in vivo.

Clinical trials of tocilizumab demonstrated its outstanding efficacy for rheumatoid arthritis, systemic juvenile idiopathic arthritis, and Castleman's disease. For patients with moderately to severely active rheumatoid arthritis, tocilizumab is now used as an innovative drug

in more than 90 countries worldwide. As a monotherapy or in combination with disease-modifying antirheumatic drugs, it has significantly suppressed disease activity and radiographic progression of joint deformity and improved daily functional activity. Tocilizumab was also approved as the first-line biologic for the treatment of systemic juvenile idiopathic arthritis in Japan, India, and the USA and for Castleman's disease in Japan and India.

In recent years, it has been found that an imbalance of new CD4-positive T cell subsets consisting of Th17 and regulatory T cells (Treg) is crucial for the development of autoimmune and chronic inflammatory diseases. As described elsewhere, dysregulated continuous production of IL-6 may be responsible for such an imbalance in which tocilizumab may be able to repair, so that it could be used for the treatment of various autoimmune and chronic inflammatory diseases. Results of pilot studies, case series, or case studies have recently suggested the possibility (Tanaka et al. 2012). These diseases include the systemic autoimmune diseases such as SLE, systemic sclerosis, polymyositis, and vasculitis syndrome; organ-specific autoimmune diseases including Crohn's disease, relapsing polychondritis, acquired hemophilia A, and autoimmune hemolytic anemia; and chronic inflammatory diseases including adult-onset Still's disease, amyloid A amyloidosis, polymyalgia rheumatica, remitting seronegative, symmetrical synovitis with pitting edema, spondyloarthritis, Behcet's disease, uveitis, and graft-versus-host diseases; and hereditary autoinflammatory syndromes (Table 1). Moreover, since it was observed that during tocilizumab treatment of patients with rheumatoid arthritis, HbA1c levels and insulin resistance indices such as HOMA-IR (homeostasis model assessment of insulin resistance) and the leptin-to-adiponectin ratio improved and serum levels reactive oxygen metabolites decreased, it can be expected that long-term tocilizumab treatment may offer protection against the progression of atherosclerosis leading to cardiovascular events. For the broad clinical indications of tocilizumab for various diseases, however, further clinical studies will be essential to determine its efficacy and safety.

On the basis of the outstanding beneficial effect of tocilizumab, other biologics of IL-6

Interleukin-6, Table 1 Clinical application of IL-6 blockade strategy for the treatment of diseases

I. Indicated diseases
1. Rheumatoid arthritis (in more than 90 countries worldwide)
2. Systemic juvenile idiopathic arthritis (in Japan, India, and the USA)
3. Polyarticular juvenile idiopathic arthritis (in Japan)
4. Castleman's disease (in Japan and India)
II. Candidate diseases
1. Autoimmune diseases
(i) Systemic lupus erythematosus
(ii) Systemic sclerosis
(iii) Polymyositis and dermatomyositis
(iv) Vasculitis syndrome (Takayasu arteritis and giant cell arteritis)
(v) Crohn's disease
(vi) Relapsing polychondritis
(vii) Acquired hemophilia A
(viii) Autoimmune hemolytic anemia
(ix) Multiple sclerosis and neuromyelitis optica
2. Chronic inflammatory diseases
(i) Adult-onset Still's disease
(ii) Amyloid A amyloidosis
(iii) Polymyalgia rheumatica
(iv) Remitting seronegative, symmetrical synovitis with pitting edema
(v) Spondyloarthritis
(vi) Behcet's disease
(vii) Uveitis
(viii) Graft-versus-host diseases
(ix) Hereditary autoinflammatory syndromes
(x) Schnitzler syndrome
(xi) Type II diabetes mellitus
3. Neoplastic diseases
(i) Multiple myeloma
(ii) Pancreatic cancer
(iii) Prostate cancer
(iv) Malignant mesothelioma

The anti-IL-6 receptor antibody, tocilizumab, has been approved as a biological drug for the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, and Castleman's disease and is expected to be used for the treatment of various other autoimmune, chronic inflammatory diseases and cancers

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blockers are also being developed. These include fully human anti-IL-6R antibody, anti-IL-6R nanobody, anti-IL-6 antibody, and anti-IL-6/anti-Fc avimer protein consisting of the IgG-binding domain fused to the N-terminus of a 3-domain IL-6-binding region, which results in a 19-kDa heterotetrameric avimer and soluble gp130-Fc fusion protein. These novel biologics are now being evaluated in clinical trials (Jones et al. 2011). It should further be pointed out that tocilizumab may not merely have a steroid-sparing effect as do other immunosuppressive drugs but may also replace corticosteroids and anti-inflammatory drugs because of its ability to normalize immunological abnormalities in addition to its anti-inflammatory activities.

Conclusion

IL-6, originally identified as a B cell differentiation factor, is a typical cytokine featuring pleiotropic and redundant activity. IL-6 is immediately generated to contribute to host defense in response to environmental stress such as infection and injury. The discovery of IL-6, IL-6 receptors, and receptor-mediated signal transduction system has made a major contribution to a better understanding of the molecular basis of pleiotropy and redundancy, which are the characteristic features of cytokines. Of similar importance is the clinical application of IL-6 blockade strategy for the treatment of intractable diseases. Based on the clarification of the pathologic role of IL-6 in disease progression, tocilizumab, a humanized anti-IL-6R antibody, was developed and clinical trials have verified that this antibody is a first-in-class biologic for the treatment of rheumatoid arthritis, systemic juvenile idiopathic arthritis, and Castleman's disease. Since dysregulation of continuous IL-6 production plays a key role in the development of various other autoimmune, chronic inflammatory diseases and even cancers, it is expected that tocilizumab will prove to be an innovative drug for the treatment of various diseases.

Cross-References

- ▶ Juvenile Idiopathic Arthritis
- ▶ Rheumatoid Arthritis, Biologics in its Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver

References

Akira S, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. Adv Immunol. 1993;54:1–78.

Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J. 2003;374(Pt 1):1–20.

Hibi M, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T. Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell. 1990;63:1149–57.

Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S, Nakajima K, Koyama K, Iwamatsu A, Tsunasawa S, Sakiyama F, Matsui H, Takahara Y, Taniguchi T, Kishimoto T. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature. 1986;324:73–6.

Hirano T, Taga T, Yasukawa K, Nakajima K, Nakano N, Takatsuki F, Shimizu M, Murashima A, Tsunasawa S, Sakiyama F, Kishimoto T. Human B-cell differentiation factor defined by an anti-peptide antibody and its possible role in autoantibody production. Proc Natl Acad Sci USA. 1987;84:228–31.

Jones SA, Scheller J, Rose-John S. Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. J Clin Invest. 2011;121:3375–83.

Kishimoto T. The biology of interleukin-6. Blood. 1989;74:1–10.

Kishimoto T. Interleukin-6: from basic sciences to medicine-40 years in immunology. Annu Rev Immunol. 2005;23:1–21.

Kishimoto T. IL-6: from its discovery to clinical applications. Int Immunol. 2010;22:347–52.

Kishimoto T, Ishizaka K. Regulation of antibody response in vitro. VII. Enhancing soluble factors for IgG and IgE antibody response. J Immunol. 1973;111:1194–205.

Kishimoto T, Akira S, Taga T. Interleukin-6 and its receptor: a paradigm for cytokines. Science. 1992;258:593–7.

Kishimoto T, Taga T, Akira S. Cytokine signal transduction. Cell. 1994;76:253–62.

Kishimoto T, Akira S, Narazaki M, Taga T. Interleukin-6 family of cytokines and gp130. Blood. 1995;86:1243–54.

Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H, Kohler G. Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature. 1994;368: 339–42.

Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S, Kishimoto T. Structure and function of a new STAT-induced STAT inhibitor. Nature. 1997;387:924–9.

Narazaki M, Yasukawa K, Saito T, Ohsugi Y, Fukui H, Koishihara Y, Yancopoulos GD, Taga T, Kishimoto T. Soluble forms of the interleukin-6 signal-transducing receptor component gp130 in human serum possessing a potential to inhibit signals through membrane-anchored gp130. Blood. 1993;82:1120–6.

Paonessa G, Graziani R, De Serio A, Savino R, Ciapponi L, Lahm A, Salvati AL, Toniatti C, Ciliberto G. Two distinct and independent sites on IL-6 trigger gp130 dimer formation and signalling. EMBO J. 1995;14:1942–51.

Tanaka T, Narazaki M, Kishimoto T. Therapeutic targeting of the interleukin-6 receptor. Annu Rev Pharmacol Toxicol. 2012;52:199–219.

Yamasaki K, Taga T, Hirata Y, Yawata H, Kawanishi Y, Seed B, Taniguchi T, Hirano T, Kishimoto T. Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor. Science. 1988;241:825–8.

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Juvenile Dermatomyositis

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Synonyms

Juvenile dermatomyositis (JDM)

Definition

Juvenile dermatomyositis (JDM) is a multisystem disease of presumably autoimmune etiology that primarily involves the skin and skeletal muscles, but may affect many other organs.

Introduction

Juvenile dermatomyositis is the most common of the juvenile idiopathic inflammatory myopathies (Feldman et al. 2008). It is a rare condition, with an incidence of 2.5–4.1 cases per million children in the United States (Mendez et al. 2003) and of 1.9 cases per million children in the United Kingdom (Symmons et al. 1995). The average age at disease onset is 7 years, but around 25 % of patients are younger than 4 years at presentation (Guseinova et al. 2011). The female-to-male ratio is 2–5:1. The disease is characterized histopathologically by perivascular inflammation, often chronic, which may lead to irreversible damage, if not appropriately treated. Over the last decades, the advances in the management of JDM, including introduction of biologic agents, have provided novel therapeutic options for use in severe cases and have dramatically decreased the disease-related morbidity and mortality.

Etiopathogenesis

Juvenile dermatomyositis is thought to be the result of the interaction between environmental factors and the host's genetic susceptibility, leading to immune dysfunction and tissue inflammation (Rider et al. 2011). The environmental trigger hypothesis is raised by reports of geographical and seasonal clustering of disease onset. Although the evidence is mostly indirect, it is also assumed that infectious agents may play a role in inducing the disease. Several children with JDM with a history of infectious illnesses prior to disease onset have been reported (Pachman et al. 2005). These infections were primarily in the upper respiratory and gastrointestinal tracts and preceded the onset of myositis by a few months. Cases of infection with parvovirus, influenza, coxsackievirus B, and group A β-hemolytic streptococci have been described. Little is known about the responsibility of noninfectious environmental factors, including stressful life events, medications,

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chemicals, excessive sun exposure, and immunizations. The genetic susceptibility is supposed to be linked to specific HLA alleles, such as B*08, DRB1*0301, DQA1*0501, and DQA1*0301, which have been observed more frequently in subjects with JDM. The activation of dendritic cells, with upregulation of genes induced by type-1 interferon in muscle tissue, seems to be pivotal for the pathogenesis. Although the pathogenetic role of type-1 interferon is still unclear, it induces an increased and sustained inappropriate expression of MHC class I molecules on the cell surface of muscle fibers, an overproduction of pro-inflammatory cytokines and chemokines, and the upregulation of both intercellular and vascular cell adhesion molecules on endothelial cells (Sugiura et al. 2000). Upregulation of MCH class I on muscle cell surface is associated to the activation of the NF-kB pathway which can, in turn, cause tissue damage. The overproduction of cytokines and adhesion molecules is crucial for the recruitment of inflammatory cells, which infiltrate the affected muscles. Cytokine polymorphisms, such as tumor necrosis factor (TNF) alpha-308 promoter polymorphism, may be risk factors in Caucasian patients (Pachman et al. 2000). Together, these immunological abnormalities are thought to contribute to the inflammatory response of JDM, and lead to dysregulation of apoptosis in myofibrils, muscle degeneration and regeneration, progressive atrophy of myofibers, and consequent functional impairment. However, the specific temporal sequence of the pathogenic events remains uncertain and, presumably, other undiscovered factors may play a role in causing the disease.

Histopathology

The mechanisms underlying vasculopathy in JDM are not yet fully explained. Histopathologically, the disease is characterized by a diffuse and often chronic, perivascular inflammation of skeletal muscle tissue (Feldman et al. 2008). Perifascicular atrophy of myofibers is typically associated with microvascular capillary pathology. Usually, swelling of the capillary endothelium and obliteration of the lumen lead to decreased number and enlargement of remaining vessels. Other changes may include disruption of myofibrils and tubular system, muscle tissue degeneration, and regeneration, which result in variation in fiber size and subsequent functional impairment. During healing, it is possible to detect an interstitial proliferation of connective tissue. A scoring system for muscle biopsy findings based on four domains of change (inflammatory, vascular, muscle fiber, and connective tissue) has been recently proposed (Wedderburn et al. 2007).

Diagnostic Criteria

The diagnosis of JDM is mainly based on the constellation of clinical and laboratory features which are part of the Bohan and Peter criteria (Table 1) (Bohan and Peter 1975a, b). These criteria include the presence of characteristic skin rashes (Gottron's papules over the extensor joint surfaces and/or the heliotrope rash over the eyelids), symmetrical proximal muscle weakness, increased serum level of one or more muscle enzymes, electromyographic demonstration of myopathy, and muscle biopsy showing characteristic histopathological changes. A diagnosis of definitive JDM requires the presence of at least one of the pathognomonic skin rashes with three other criteria; the classic rash plus two other criteria are required for a diagnosis

Juvenile Dermatomyositis, Table 1 Diagnostic criteria for juvenile dermatomyositis

I.	Pathognomonic skin rash (Gottron's papules over the extensor joint surfaces and/or heliotrope rash over the eyelids)
II.	Symmetrical proximal muscle weakness
III.	Increased serum level of one or more muscle enzymes
IV.	Electromyographic demonstration of the characteristic myopathy
V.	Muscle biopsy showing typical histopathologic changes

The diagnosis of juvenile dermatomyositis requires the presence of the pathognomonic skin rash with 3 of the other 4 criteria

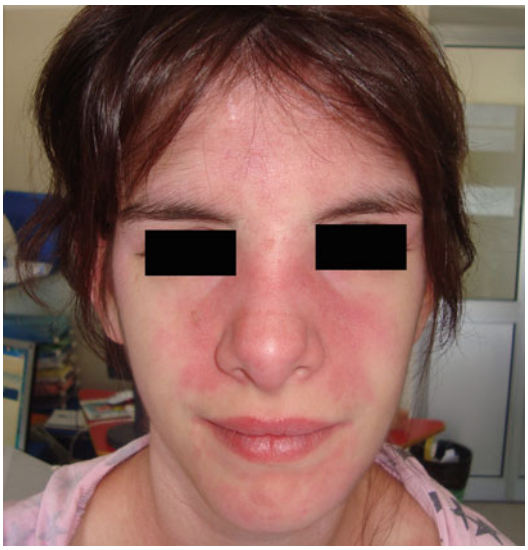
of probable JDM. An international survey has shown that a sizable number of pediatric rheumatologists do not use electromyography or muscle biopsy to pursue the diagnosis and consider magnetic resonance imaging (MRI) as one of the most important additional diagnostic methods (Brown et al. 2006).

Disease Onset and Course

The onset of JDM is quite variable, with some patients experiencing the insidious development of progressive muscle weakness and skin rash, and others having a more acute onset with fever, profound muscle weakness, widespread cutaneous manifestations, and, occasionally, ulcerative lesions. The disease course is heterogeneous as well, and may range from a monocyclic course, with good response to treatment and full recovery within 2 years after diagnosis without relapse, to a chronic polycyclic or continuous course, with relapsing-remitting disease, or persistently active disease for longer than 2 years after diagnosis, and a greater likelihood of development of disease-related complications, such as calcinosis or lipodystrophy (Rider et al. 2011).

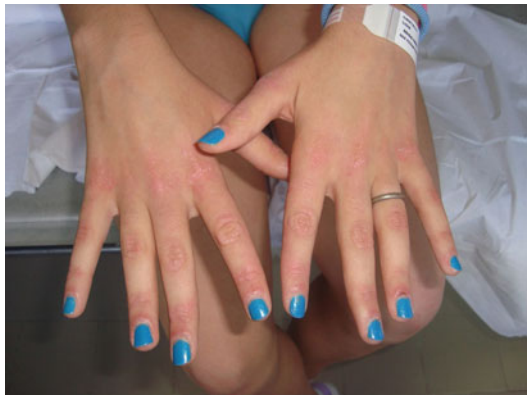
Clinical Manifestations

The three most typical, and most common, cutaneous manifestations of JDM are heliotrope discoloration of the upper eyelids, Gottron's papules, and periungual erythema with capillary loop abnormalities (Vitale et al. 2004). The heliotrope rash occurs over the upper eyelids and the periorbital region as a dusky, violaceous-reddish purple erythema, which may be associated with a malar rash resembling that of systemic lupus erythematosus (SLE) in its distribution, though less well demarcated (Fig. 1), or to a more confluent erythema involving the entire face. The malar rash of JDM is also distinguished from that of juvenile SLE by the involvement of nasolabial folds, that is typically absent in lupus. In the acute phase, the heliotrope rash is often accompanied by edema of the eyelids or the face or both, resulting

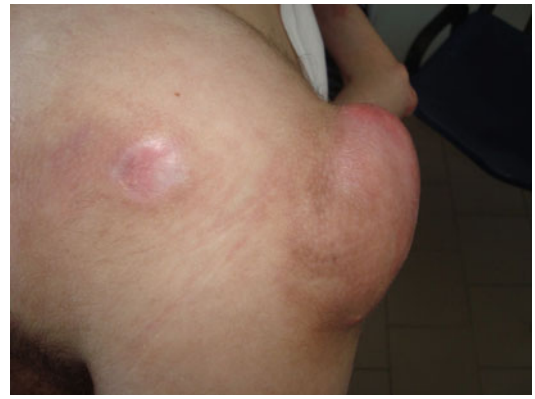


Juvenile Dermatomyositis, Fig. 1 Malar erythema

in sensation of tightness. JDM rash is photosensitive in up to 30 % of patients. Capillary telangiectasias along the lid margin may persist long after the other signs and symptoms of disease activity have resolved. Gottron's papules are pink-red to violaceous, flat-topped papules and plaques that are located most commonly over the extensor surfaces of the proximal interphalangeal joints of the hands and less so over the metacarpophalangeal and distal interphalangeal joints (Fig. 2). The skin over the thumbs is rarely, if ever, affected. The extensor surfaces of the elbows and knees and, less frequently, the malleoli and the vertebral apophyses may be involved as well. A macular erythematous or violaceous eruption may involve discrete areas of the body, producing specific signs: the "V-sign" for the V of the neck and upper chest; the "shawl-sign" for the nape of the neck, upper back, and posterior aspect of the shoulders. In the most active and severe forms of JDM, the cutaneous rash may extend considerably and lead to total body erythema (erythroderma). Nailfold capillary abnormalities due to a widespread capillary vasculopathy, which is the underlying pathologic lesion of JDM, and periungual erythema are detectable in 50 % to 100 % of patients with JDM. The most common abnormalities are capillary dropout, dilatation of



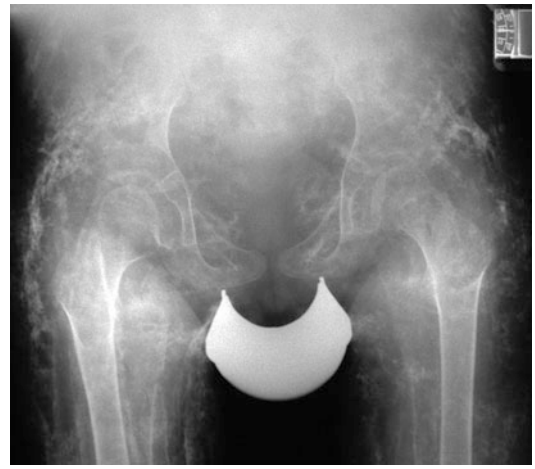
Juvenile Dermatomyositis, Fig. 2 Gottron's papules over the knuckles



Juvenile Dermatomyositis, Fig. 3 Large tumorous calcium deposit overlying the left hip region

isolated loops, thrombosis, and hemorrhage. Arborized clusters of giant capillary loops, which are secondary to post-ischemic neovascularization, are distinctive of JDM. These changes have been correlated with a more severe disease course or with the development of cutaneous ulceration or calcinosis, and have been found to abate with disease remission. Cutaneous ulceration affects less than 10 % of children with JDM, but may predict a severe course of illness.

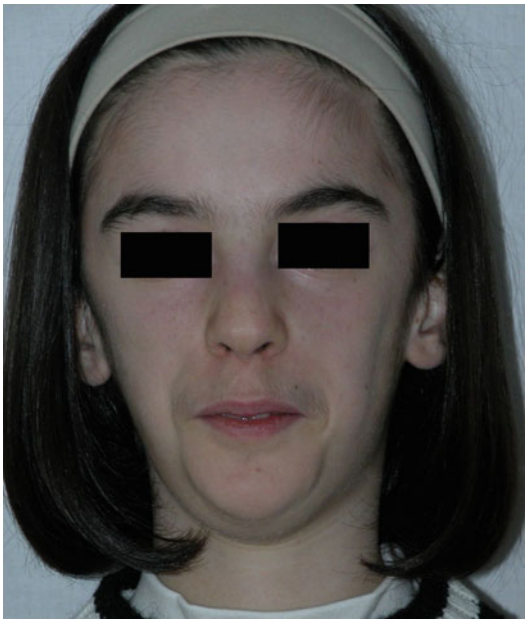
Muscle inflammation usually results in weakness of proximal muscles, especially the limb girdle musculature (Rider et al. 2011). In addition, abdominal, back, and anterior neck flexor muscles are often affected and lead to protrusion of abdomen, inability to hold the head upright and to maintain a sitting posture. The child may experience difficulties in walking, or inability to dress or climb stairs. Physical examination shows symmetrical muscle weakness, particularly pronounced in the hip muscles, neck flexors, and abdominal musculature. Muscles may be tender, painful, and develop contractures. Edema of the overlying subcutaneous tissue of affected muscles is not uncommon. Gower's sign is often present. Palatal, hypopharyngeal, and pharyngeal muscles may be involved in case of severe disease, resulting in difficulty in swallowing, dyspnea, and dysphagia. Sometimes patients may complain of arthralgia/arthritis. Gastrointestinal vasculitis may be heralded by the occurrence of hematemesis and melena, and may cause ulceration of mucosa



Juvenile Dermatomyositis, Fig. 4 Pelvis radiograph showing extensive dystrophic calcification

and perforation. Severe cardiac involvement such as pericarditis and myocarditis, and interstitial pneumonitis are rare complications of JDM.

Calcinosis is an important complication and may occur in more than 20 % of children with JDM (Figs. 3 and 4) (Pachman et al. 2005; Ravelli et al. 2010). Although it develops more frequently as a late manifestation of illness, it may present at any time. Calcinosis has been associated with a longer duration of active disease (Ravelli et al. 2010). Pressure points are more commonly involved in the form of nodules and plaques, which may produce local erythema or skin ulceration and cellulitis. Calcinosis may



Juvenile Dermatomyositis, Fig. 5 Facial lipodystrophy

also extend along muscles and fascial planes or even form an exoskeleton, particularly when active myositis is severe or inappropriately treated. Regression is possible spontaneously or through extrusion.

Acquired lipodystrophy is seen in approximately 10 % of cases (Ravelli et al. 2010) and is characterized by a progressive loss of subcutaneous and visceral fat that may be generalized, partial (affecting mainly extremities), or focal (often involving sites of calcinosis) (Fig. 5). This condition is commonly associated with insulin resistance, acanthosis nigricans, abnormal glucose tolerance, dyslipidemia, hyperandrogenism, and amenorrhea.

The main clinical manifestations of JDM are summarized in Table 2.

Juvenile Dermatomyositis, Table 2 Clinical features of juvenile dermatomyositis

Constitutional	Fever Anorexia Fatigue Lethargy
Cutaneous	Heliotrope rash Malar or facial rash Gottron's papules Nailfold capillary changes Edema Cutaneous or mouth ulcerations Calcinosis Lipodystrophy
Musculoskeletal	Proximal muscle weakness or myalgia Arthralgia or arthritis Joint contractures
Pulmonary	Dysphonia Dyspnea
Gastrointestinal	Dysphagia Gastrointestinal symptoms

Laboratory Abnormalities

At disease onset, muscle inflammation is generally accompanied by the elevation of serum level of muscle enzymes, including creatinine kinase (CK), lactate dehydrogenase, transaminases, and aldolase. However, muscle enzymes are not particularly sensitive, as more than 20 % of patients have normal CK at diagnosis (Ramanan and Feldman 2005). Furthermore, muscle enzymes frequently become normal with therapy, even during active disease or disease exacerbations. Of the remaining serological abnormalities, autoantibodies, mainly antinuclear antibodies (ANAs) and anti-aminoacyl transfer RNA synthetases (anti-Jo1), have been reported with variable frequency.

Disease Assessment

Assessment of the extent and severity of muscle inflammation is of pivotal importance in evaluating disease activity and response to treatment in children with JDM, and muscle strength is the principal clinical parameter to estimate muscle involvement. Manual muscle testing (MMT) by Kendall is the most widely used method for muscle strength measurement in therapeutic trials and assesses a representative subset of eight proximal, distal, and axial muscles tested unilaterally, in the dominant side of the patient. Assessment of physical function in JDM is based on the estimation of muscle function,

which includes evaluation of muscle endurance and fatigue. The tool commonly used for assessment of muscle function is the Childhood Myositis Assessment scale (CMAS), which measures the ability of the patients in performing 14 maneuvers. Overall disease activity is assessed through the Disease Activity Score (DAS) and the Myositis Disease Activity Assessment VAS (MYOACT). Physical function can be evaluated through the Childhood Health Assessment Questionnaire (C-HAQ), whereas health-related quality of life can be determined through the parent version of the Child Health Questionnaire (CHQ). Cumulative damage is estimated based on the Myositis Damage Index (MDI). MRI is able to detect muscle inflammation as edema. It is also useful in selecting a site to perform electromyography or muscle biopsy, which helps reduce the rate of false-negative results. MRI is also a valid indicator of muscle disease activity and may help distinguish activity from damage. The tools used in the assessment of JDM have been reviewed recently (Rider 2002; Ravelli et al. 2006).

Management

So far, there have been no randomized controlled trials of any medications for children with JDM. As a result, disease management remains largely empiric and only based on observational studies and clinical experience (Feldman et al. 2008; Rider et al. 2011). There is a common agreement that corticosteroids are the mainstay of treatment, although the regimens for such medications in induction, maintenance, or tapering are variable among physicians and depend on clinical experience. High doses of steroids are frequently administered intravenously at the time of diagnosis because of the potentially poor gastrointestinal absorption due to the vasculopathy. This approach is usually followed by oral administration of steroids coupled with second-line drugs, namely methotrexate (15–20 mg/m^2 weekly sc) or cyclosporine (3–5 mg/kg per day orally), which are given as steroid-sparing agents. It has been suggested that early administration of methotrexate may reduce the risk of disease-related morbidity, particularly the development of calcinosis. Once disease control is achieved, corticosteroids are tapered slowly until discontinuation; this frequently takes 2 years or more. A low dose is usually maintained for a long period to prevent disease flares. In severe or refractory cases and in the presence of specific clinical features, patients may benefit from other medications. Hydroxychloroquine (3–5 mg/kg per day orally) is frequently used for cutaneous manifestations and monthly intravenous immunoglobulin has proven to be effective for resistant skin rash. Azathioprine (1–2 mg/kg per day orally) and cyclophosphamide (2 mg/kg per day orally) are recommended in case of methotrexate or cyclosporine intolerance or failure, or as adjunctive treatments for aggressive disease. Little is known about the role of biologic agents in the management of JDM. Although use of B-cell-depleting therapy with rituximab is not supported by strong scientific evidence, anecdotal data suggest it can be a promising therapeutic option for refractory cases. A recent randomized clinical trial of rituximab in refractory adult and pediatric myositis patients did not show a significant difference in the time to achieving the definition of improvement between patients who received rituximab early or late. However, 83% of adult and juvenile myositis patients met the definition of improvement (Oddis et al. 2013). Photoprotective agents and topical medications (tacrolimus, steroids) may be effective in localized skin rashes. Physiotherapy is often needed to avoid functional impairment due to calcinosis or joint contractures, and to shorten the time to recover full muscle strength.

Outcome and Prognosis

Prior to the introduction of corticosteroids in the 1960s, almost one-third of patients with JDM died, one-third were left with permanent disabilities, and only one-third recovered without complications (Huber and Feldman 2005). Since then, the mortality has decreased to less than 2 %, and there has been considerable improvement in

functional outcome. However, there are still many patients who are refractory or respond suboptimally to current treatments and are at risk of developing irreversible damage from the disease activity or its treatment. A recent large multinational study (Ravelli et al. 2010) has shown that patients with JDM had favorable functional outcomes, with only a few experiencing severe muscle weakness or physical disability at follow-up assessment. Furthermore, most patients had a good quality of life and were satisfied with the outcome of the illness. However, many patients continued to have chronic disease activity and had evidence of cumulative damage. Furthermore, a chronic course of the illness was the most consistent predictor of a poorer long-term outcome in terms of muscle weakness, continued disease activity, cumulative damage, and functional impairment. Development of calcinosis and lipodystrophy were associated with greater duration of active disease. These findings underscore the critical need for treatments and treatment strategies that have the ability to better control disease activity over time and to reduce the development of non-reversible organ damage.

Cross-References

- ▶ Dermatomyositis, Skin
- ▶ Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies
- ▶ Myositis, Pathogenesis

References

Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med. 1975a;292(7):344–7.

Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med. 1975b;292(8):403–7.

Brown VE, Pilkington CA, Feldman BM, Davidson JE; Network for juvenile dermatomyositis, Paediatric Rheumatology European Society (PReS). An international consensus survey of the diagnostic criteria for juvenile dermatomyositis (JDM). Rheumatology. 2006;45(8):990–3.

Feldman BM, Rider LG, Reed AM, Pachman LM. Juvenile dermatomyositis and other idiopathic inflammatory myopathies of childhood. Lancet. 2008;371(9631):2201–12.

Guseinova D, Consolaro A, Trail L, Ferrari C, Pistorio A, Ruperto N, Buoncompagni A, Pilkington C, Maillard S, Oliveira SK, Sztajnbok F, Cuttica R, Corona F, Katsicas MM, Russo R, Ferriani V, Burgos-Vargas R, Solis-Vallejo E, Bandeira M, Baca V, Saad-Magalhaes C, Silva CA, Barcellona R, Breda L, Cimaz R, Gallizzi R, Garozzo R, Martino S, Meini A, Stabile A, Martini A, Ravelli A. Comparison of clinical features and drug therapies among European and Latin American patients with juvenile dermatomyositis. Clin Exp Rheumatol. 2011;29(1):117–24.

Huber A, Feldman BM. Long-term outcomes in juvenile dermatomyositis: how did we get here and where are we going? Curr Rheumatol Rep. 2005;7(6):441–6.

Mendez EP, Lipton R, Ramsey-Goldman R, Roettcher P, Bowyer S, Dyer A, Pachman LM. Juvenile DM Registry Physician Referral Group. US incidence of juvenile dermatomyositis, 1995–1998: results from the National Institute of Arthritis and Musculoskeletal and Skin Diseases Registry. Arthritis Rheum. 2003;49(3):300–5.

Oddis CV, Reed AM, Aggarwal R, Rider LG, Ascherman DP, Levesque MC, Barohn RJ, Feldman BM, Harris-Love MO, Koontz DC, Fertig N, Kelley SS, Pryber SL, Miller FW, Rockette HE. RIM Study Group. Rituximab in the treatment of refractory adult and juvenile dermatomyositis and adult polymyositis: a randomized, placebo-phase trial. Arthritis Rheum 2013;65(2):314–24.

Pachman LM, Liotta-Davis MR, Hong DK, Kinsella TR, Mendez EP, Kinder JM, Chen EH. TNFalpha-308A allele in juvenile dermatomyositis: association with increased production of tumor necrosis factor alpha, disease duration, and pathologic calcifications. Arthritis Rheum. 2000;43(10):2368–77.

Pachman LM, Lipton R, Ramsey-Goldman R, Shamiyeh E, Abbott K, Mendez EP, Dyer A, Curdy DM, Vogler L, Reed A, Cawkwell G, Zemel L, Sandborg C, Rivas-Chacon R, Hom C, Ilowite N, Gedalia A, Gitlin J, Borzy M. History of infection before the onset of juvenile dermatomyositis: results from the National Institute of Arthritis and Musculoskeletal and Skin Diseases Research Registry. Arthritis Rheum. 2005;53(2):166–72.

Ramanan AV, Feldman BM. The role of muscle enzymes in JDM. Pediatr Rheumatol Online J. 2005;3:1–4.

Ravelli A, Ruperto N, Trail L, Felici E, Sala E, Martini A. Clinical assessment in juvenile dermatomyositis. Autoimmunity. 2006;39(3):197–203.

Ravelli A, Trail L, Ferrari C, Ruperto N, Pistorio A, Pilkington C, Maillard S, Oliveira SK, Sztajnbok F, Cuttica R, Beltramelli M, Corona F, Katsicas MM, Russo R, Ferriani V, Burgos-Vargas R, Magni-Manzoni S, Solis-Valleoj E, Bandeira M, Zulian F, Baca V, Cortis E, Falcini F, Alessio M, Alpigiani

MG, Gerloni V, Saad-Magalhaes C, Podda R, Silva CA, Lepore L, Felici E, Rossi F, Sala E, Martini A. Long-term outcome and prognostic factors of juvenile dermatomyositis: a multinational, multicenter study of 490 patients. Arthritis Care Res. 2010;62(1):63–72.
Rider LG. Outcome assessment in the adult and juvenile idiopathic inflammatory myopathies. Rheum Dis Clin N Am. 2002;28(4):935–77.
Rider LG, Lindsley CB, Cassidy JT. Juvenile dermatomyositis. In: Cassidy JT, Petty RE, Laxer RM, Lindsley CB, editors. Textbook of pediatric rheumatology. Sixth Edition. Philadelphia: Elsevier Saunder; 2011. p. 375–413.
Sugiura T, Kawaguchi Y, Harigai M, Takagi K, Ohta S, Fukasawa C, Hara M, Kamatani N. Increased CD40 expression on muscle cells of polymyositis and dermatomyositis: role of CD40-CD40 ligand interaction in IL-6, IL-8, IL-15, and monocyte chemoattractant protein-1 production. J Immunol. 2000;164(12):6593–600.
Symmons DP, Sills JA, Davis SM. The incidence of juvenile dermatomyositis: results from a nation-wide study. Br J Rheumatol. 1995;34(8):732–6.
Vitale A, Trail L, Felici E, Traverso F, Martini A, Ravelli A. Cutaneous manifestations of juvenile systemic lupus erythematosus and juvenile dermatomyositis. Pediatr Rheumatol Online J. 2004;2(5).
Wedderburn LR, Varsani H, Li CK, Newton KR, Amato AA, Banwell B, Bove KE, Corse AM, Emslie-Smith A, Harding B, Hoogendijk J, Lundberg IE, Marie S, Minetti C, Nennesmo I, Rushing EJ, Sewry C, Charman SC, Pilkington CA, Holton JL. International consensus on a proposed score system for muscle biopsy evaluation in patients with juvenile dermatomyositis: a tool for potential use in clinical trials. Arthritis Rheum. 2007;57(7):1192–201.

Juvenile Diseases: SLE in Children

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Synonyms

Juvenile-onset SLE; Pediatric SLE

Definition

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder that can affect any organ of the human body. Presentation and clinical courses are highly variable which is why 11 diagnostic criteria were introduced and 4 must be fulfilled to make a diagnosis of SLE (Tsokos 2011; Hochberg 1997).

The pathophysiology of SLE is not completely understood; genetic, hormonal, and environmental factors, all contribute to the development of the disease (Hedrich et al. 2011). Women are affected more frequently with an overall female to male ratio of 10:1 (Tsokos 2011). Approximately 10–20 % of all SLE manifestations occur in the pediatric age group (before 16 years of age) and are classified as pediatric SLE (or juvenile-onset SLE) (Ardoin and Schanberg 2012; Brunner et al. 2011). Cases with a disease onset before 5 years of age, referred to as early-onset SLE, are extremely uncommon and appear to be more severe when compared to other forms of SLE (Hedrich et al. 2011; Zulian et al. 2008). Interestingly, the gender distribution varies significantly in the pediatric age group with an almost equal gender distribution in the first decade of life and development of the "classical" female predominance during the second decade (female:male 4:1 in the second decade, 10:1 in cases >20 years) (Mina and Brunner 2011; Brunner et al. 2011). Disease onset in the pediatric age group peaks around 12–14 years. This implies that hormonal influences may be more important for the pathophysiology of adult-onset SLE when compared to pediatric SLE. Though symptoms and diagnostic criteria for pediatric and adult-onset SLE are generally identical, pediatric SLE is characterized by a more acute and aggressive disease onset, higher chronic disease activity, and a higher frequency of severe organ involvement, including the kidneys, the central nervous system (CNS), and hematologic manifestations (Hedrich et al. 2011; Zulian et al. 2008; Ardoin and Schanberg 2012; Hersh et al. 2010; Livingston et al. 2011, 2012).

The differences in gender distribution, disease activity, severity, and outcome in pediatric SLE,

Juvenile Diseases: SLE in Children, Table 1 Organ involvement *at the time of presentation* and outcome in adult vs. early-onset SLE (N/A: no data available) (Zulian et al. 2008; Hedrich et al. 2011; Gaubitz and Schotte 2005)

Organ involvement	Adult SLE (European Caucasian)	Early-onset SLE (Mostly Caucasian)
Kidneys	16 %	~93 %
CNS	12 %	~33 %
Serositis	17 %	N/A
Mucous membranes	11 %	N/A
Skin	49 %	60 %
Raynaud's	18 %	N/A
Musculoskeletal system	69 %	~33 %
Lymphadenopathy	7 %	~27 %
Fevers	N/A	60 %
Irritability	N/A	40 %
Survival rate	86 % (10-year survival)	~67 % (after months to >20 years)

combined with the increased prevalence of genomic mutations and risk alleles in pre-pubertal autoimmune disorders (Haas 2010), suggest that a number of potentially distinct disorders may be contained within the term "pediatric SLE."

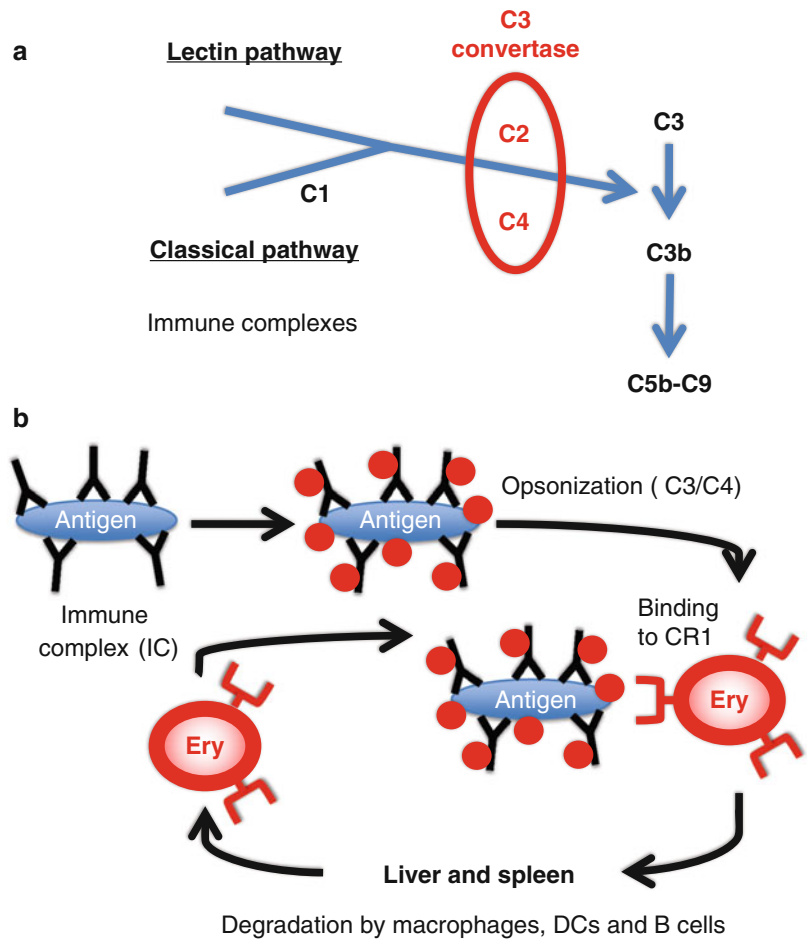
Early-Onset SLE

Early-onset SLE involves extremely rare and heterogeneous disease presentations with more variable and severe symptoms as compared to any other form of SLE (Hedrich et al. 2011; Zulian et al. 2008; Brunner et al. 2011; Ardoin and Schanberg 2012). To our knowledge, only 15 patients have been reported, some still within the first year of life at disease onset. A review of the available literature suggests more severe disease courses in the early-onset SLE age group when compared to adult-onset SLE cases (Table 1) (Hedrich et al. 2011). Early-onset SLE patients seem to develop more severe organ manifestations with a higher rate of complications at the time of diagnosis. More than 90 % of reported patients presented with kidney involvement, and one third suffered from CNS disease that manifested with calcifications, seizures, or cerebral hemorrhages. Survival rates were markedly reduced when compared to other age groups (Table 1) (Zulian et al. 2008; Hedrich et al. 2011; Gaubitz and Schotte 2005). However, epidemiological and predictive information in the early-onset SLE age group needs to be handled with caution secondary to the lack of clinical studies or surveys, the very small number of reported cases, the potential of under-diagnosing milder cases in this young age group, and the potential of an over-representation of severe cases in the literature.

Unfortunately, all early-onset SLE patients have been reported as single case reports or small case series and genetic data is lacking (Hedrich et al. 2011; Zulian et al. 2008). However, it appears likely that hormonal and environmental factors, which in the case of adult SLE accumulate over time, play a less central role in the pathophysiology of early-onset SLE, and that genetic factors are largely responsible for the disease.

Monogenic SLE: Complement Deficiencies

Several rare genetic disorders result in lupus-like disease. Mutations within the class III or complement region of the human histocompatibility (MHC) complex on chromosome 6 can result in SLE or lupus-like disease (Truedsson et al. 2007). The genes encoding for the complement factors C4A, C4B (a duplication of C4A), factor B and C2 are located within that region. Another example for monogenic SLE can be found in the gene encoding for C1q (chromosome 1). Regardless of not being located in the MHC cluster (Truedsson et al. 2007; Flierman and Daha 2007; Atkinson and Yu 2011), C1q is essential for the classical complement activation pathway, and mutations in the C1 coding region frequently result in autoimmune phenomena and lupus-like disease. The risk for developing SLE or lupus-like disorders varies between the reported defects: 90 % of the patients with C1q deficiency develop SLE, while 60 % of the individuals with



Juvenile Diseases: SLE in Children, Fig. 1 Both the lectin and the classical complement activation pathways are essential for the clearance of immune complexes (**a**). The complement system is involved in the opsonization and removal of immune complexes. Activated complement factors C3b and C4b opsonize immune complexes which results in the binding of opsonized immune complexes to complement receptors (CR1) on erythrocytes and subsequent degradation in the liver or spleen by antigen-presenting cells (**b**)

C4 deficiencies and 30 % of the patients with C2 deficiency develop SLE-like disorders. Complement factor deficiencies down-stream of C3 are less likely to manifest as SLE, but do result in severe infections (Truedsson et al. 2007; Flierman and Daha 2007; Atkinson and Yu 2011).

The development of lupus-like disorders results from the central involvement of C1, C2, and C4 in the early stages of complement activation. C2 and C4 are both required for the initiation of the lectin pathway and the classical activation pathway. C1 complement factors are required for sufficient complement activation in the classical pathway (Fig. 1a). In response to activation, C2 and C4 together form the so-called C3 convertase that is responsible for C3 cleavage and the activation of down-stream complement factors (Atkinson and Yu 2011).

The observation that a large percentage of individuals with deficiencies of "early" complement factors of the classical and/or the lectin pathway develop SLE or lupus-like disorders together with findings in complement-deficient mice led to the conclusion that these defects result in an accumulation of necrotic or apoptotic material and an increase in circulating and tissue immune complexes. Immune complex deposition in vessels and organs results in tissue damage and systemic inflammation (Botto 1998). Under physiological conditions, complement factors C3b and C4b are involved in the opsonization of immune complexes. Opsonization of immune complexes allows the binding to complement

receptors on erythrocytes and subsequent immune complex degradation in the liver or spleen by antigen-presenting cells (Fig. 1b). A lack of activated complement factors C3b and C4b results in inappropriate formation and impaired handling of immune complexes. The subsequent precipitation of immune complexes in the vascular bed or organs and tissues results in local inflammation and vasculitis. Furthermore, inappropriately removed intracellular antigens from apoptotic or necrotic cells may be recognized as "foreign" and become autoantigens, accelerating autoimmune pathology (Truedsson et al. 2007; Flierman and Daha 2007; Atkinson and Yu 2011; Hauck et al. 2011).

Genetic Susceptibility and the Hypothesis of "Oligogenic" SLE

The case for a strong genetic component of SLE is emphasized by an increased risk for the development of SLE with a positive family history (30-fold) and high concordance rates between monozygotic (55 %) and dizygotic twins (5 %) (Tsokos 2011; Mina and Brunner 2011; Brunner et al. 2011). In addition to the aforementioned complement deficiencies, which represent distinct monogenic forms of SLE, several nucleotide exchanges in coding or noncoding regions of lupus susceptibility genes have been reported (Box 1). Some of which can, by themselves, cause lupus-like symptoms that are usually limited to single organ manifestations.

Examples are mutations in the 3′ DNA repair exonuclease TREX1, which cause chilblain lupus erythematosus (CLE) (Lee-Kirsch et al. 2007a). CLE is a chronic disorder that is characterized by inflammatory skin lesions on the fingers, toes, ears, nose, and cheeks in response to temperature change or cold and damp climate (Hedrich et al. 2008; Lee-Kirsch et al. 2007a). While spontaneous cases in middle-aged women are most common, familial cases affect both genders and are caused by missense mutations in *TREX1*. A subset of CLE patients (18–20 %) later on develop SLE and 0.5–3 % of SLE patients in large cohorts in Europe and the USA exhibit mutations in *TREX1* (Lee-Kirsch et al. 2007b; Namjou et al. 2011). Two pathophysiological mechanisms have been proposed for TREX1 mutations: (1) mutations in regions with exonuclease activity (which occur in familial CLE) may result in an accumulation of cytoplasmic single-stranded DNA that results in increased IFN-α signaling, and (2) mutations in regions without exonuclease activity (as those found in the SLE cohorts) may interfere with TREX1's ability to associate with the SET complex, thus affecting granzyme A–mediated caspase-1-independent cell death (Aringer et al. 2012).

Given that mutations in single SLE susceptibility genes can cause organ manifestations that are part of the clinical picture of SLE (e.g., *TREX1* in CLE) (Lee-Kirsch et al. 2007a, b), and familial clusters of SLE are not uncommon resulting in an increased risk for children and their siblings (especially twins) (Mina and Brunner 2011; Brunner et al. 2011), one can hypothesize that the combination of such risk alleles may result in SLE (Aringer et al. 2012). In such cases, epigenetic and/or hormonal factors may be less important than in "classical" SLE of the adult age group (Hedrich and Tsokos 2011). The combination of genomic variants in susceptibility genes may result in early disease onset and the almost equal gender distribution in the first decade of life. At this point, genetic studies in pediatric SLE cohorts are, for the most part, limited to the confirmation of the findings in adult SLE and cannot explain the differences in disease presentation, activity, chronicity, and outcome (Brunner et al. 2011; Ardoin and Schanberg 2012; Hedrich et al. 2011). However, findings from larger cohorts suggest that pediatric autoimmune disorders (mostly juvenile idiopathic arthritis) have a stronger genetic basis when compared to adult disease (rheumatoid arthritis) (Haas 2011). In light of the limited data on pediatric SLE, the hypothesis of a stronger genetic background in pediatric SLE remains somewhat speculative and warrants genetic studies in large cohorts, including patients with early-onset, pediatric, and adult-onset SLE.

Box 1: Risk Alleles in the Pathophysiology of SLE (Tsokos 2011; Crispín et al. 2010)

Gene variants that are associated with increased susceptibility to SLE affect immune signaling pathways. This is an exemplary overview of several candidate genes, involved in:

1. The clearance and processing of immune complexes through, e.g.,
   (a) The Fc Receptors for immunoglobulin G (FcγR) which are involved in immune complex removal and antibody-dependent immune responses
   (b) Complement factors C1q (SNPs or complete deficiency), C2 and C4 (deficiency)
   (c) C-reactive protein (CRP) that is involved in immune activation and the clearance of necrotic and apoptotic debris
2. Toll-like receptor/Type I interferon signaling through, e.g.,
   (a) Single base exchanges (SNPs) in *STAT4* that are associated with SLE and/or more severe outcomes
   (b) *TREX1* mutations affecting its exonuclease activity or its association with the SET complex, resulting in defective caspase 1-independent apoptosis
   (c) TLR7 and TLR9 variants that affect immune complex clearance and IFN-α responses
3. The transduction of immune signals through, e.g.,
   (a) *IL10* promoter polymorphisms, which are associated with increased IL-10 expression and B cell activation in SLE.
   (b) *BANK1* variants that may increase calcium flux in B cells.
   (c) *PP2A* polymorphisms in the first intron, which may result in increased PP2A expression and down-stream activation of protein kinases.
   (d) Variants in the interferon regulatory factor-5 (*IRF5*), a transcription factor in the type I IFN pathway. *IRF5* variants are furthermore associated with macrophage activation syndrome in pediatric SLE.

Post-pubertal SLE

Pediatric SLE patients that manifest after the onset of puberty usually present very similarly or identically when compared with adult-onset SLE patients. Comparably to adult-onset SLE, the ACR diagnostic criteria for SLE from 1997 reflect the clinical spectrum with its wide range of organ manifestations (Hersh et al. 2010; Brunner et al. 2011; Ardoin and Schanberg 2012; Tsokos 2011). Furthermore, the gender distribution changes from an almost equal ratio between boys and girls in early-onset SLE to the "classical" picture with a female predominance (4:1 in the second decade and 10:1 after the 20th year of age) (Mina and Brunner 2011; Brunner et al. 2011; Tsokos 2011). Thus, the general manifestation and epidemiology of post-pubertal pediatric SLE generally reflect the "classical" picture of adult-onset SLE. However, a number of studies demonstrated a more acute and severe onset of post-pubertal pediatric SLE followed by chronically active disease courses, resulting in more severe organ damage and increased mortality rates (Hersh et al. 2010; Brunner et al. 2011; Ardoin and Schanberg 2012; Silverman and Eddy 2011). To this point, differences in the pathophysiology of pediatric and adult-onset SLE remain to be elucidated. It remains speculation whether differences in the contribution of genetic predisposition vs. environmental and/or hormonal influences account for these distinct differences in disease severity and outcome.

Treatment of Pediatric SLE

Treatment of pediatric SLE is complex and less standardized when compared to adult-onset SLE. The lack of large controlled multicenter trials in the pediatric age group complicates treatment decisions. Furthermore, potential toxic and treatment-related side effects need to be considered in the context of physical and mental development. Regardless of these concerns, pediatric patients require higher steroid doses and immune

Juvenile Diseases: SLE in Children, Table 2 Treatment considerations in pediatric SLE

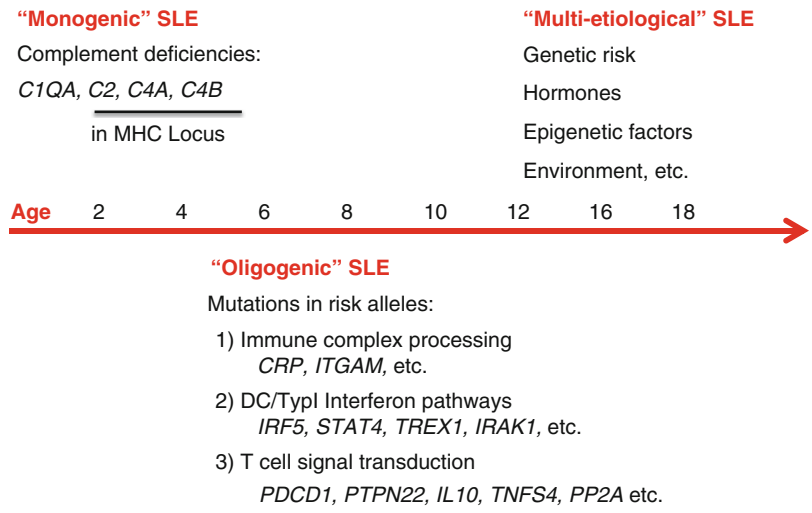
Therapeutic agents	Used for	Special considerations
NSAIDs	Mild disease	Musculoskeletal involvement
Anti-malaria drugs	Mild disease	Cutaneous involvement, vasculitis
		Arthritis
		Autoinflammation: TLR interference
Corticosteroids	Control of acute disease	Glomerulonephritis (Fauci/NIH, Euro-lupus)
	Treatment induction	Liver disease
	Low-dose maintenance	Vasculitis
DMARDs		
Azathioprine Mycophenolate	Vasculitis Glomerulonephritis Liver disease	Glomerulonephritis induction and/or maintenance therapy (Fauci/NIH and Euro-lupus protocols) >Depending on type and severity of kidney disease
Methotrexate	Arthritis, skin disease (CNS disease)	Cave: Not in kidney or liver disease
Cyclophosphamide	Vasculitis Glomerulonephritis CNS disease	Glomerulonephritis induction therapy (Fauci/NIH and Euro-lupus protocols) >Depending on type and severity of kidney disease
Biologicals		Clinical data is very limited. The use of biologicals can only be recommended on an individual basis and in a center experienced in the treatment of pediatric SLE patients
Anti-CD20	Complement deficiencies	
	Treatment-resistant SLE	
Anti-TNF	Treatment-resistant SLE	
	Vasculitis	

suppressants, considering the more aggressive course of pediatric SLE (Hersh et al. 2010; Brunner et al. 2011; Ardoin and Schanberg 2012; Silverman and Eddy 2011).

For certain subgroups of pediatric SLE patients, more or less target-directed forms of treatment are available. Since complement deficiencies result in altered clearance of apoptotic and necrotic material, defective clearance of circulating and organ immune complexes, and subsequent B lymphocyte activation, B cell depletion with recombinant antibodies has proven effective and may be superior to other immune suppressants (Atkinson and Yu 2011; Hauck et al. 2011). Limited but consistent reports suggest that treatment decisions can be based on organ involvement and disease severity (Table 2). The translation of scientific evidence into clinical practice will allow more target-directed treatment with reduced toxic effects. This will also increase the compliance of pediatric patients, which will further improve the clinical outcome (Mina and Brunner 2011; Silverman and Eddy 2011).

Conclusions

Pediatric SLE takes a more acute, aggressive, and chronically active course when compared to adult-onset SLE. Age-related differences in organ involvement, disease course, and outcome as well as marked differences in the gender distribution strongly suggest varying pathomechanisms within the entity pediatric SLE. Monogenic disorders usually manifest early in childhood. It appears likely that the combination of two or more risk alleles can result in



Juvenile Diseases: SLE in Children,
Fig. 2 Hypothetical model of the influence of genetic, hormonal, and environmental factors to the pathophysiology of pediatric SLE

SLE or lupus-like manifestations in childhood in the absence or presence of hormonal and environmental factors (Fig. 2). Further studies are warranted, investigating genetic predisposition for the development of SLE. A better understanding of the pathophysiological mechanisms in early-onset, pediatric, and adult-onset SLE will help to establish more individually tailored, target-directed, and effective forms of treatment with reduced toxicity.

Cross-References

- Autoinflammatory Diseases
- Complement in Rheumatic Diseases
- Complement Regulation in the Kidney
- Juvenile Idiopathic Arthritis
- Lupus Nephritis, Diagnosis and Treatment
- Novel Targets in Systemic Lupus Erythematosus
- Systemic Lupus Erythematosus, Gender and Hormone Influences
- Systemic Lupus Erythematosus, Genetics
- Systemic Lupus Erythematosus, Pathogenesis
- Systemic Lupus Erythematosus, Treatment
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Ardoin SP, Schanberg LE. Paediatric rheumatic disease: lessons from SLE: children are not little adults. Nat Rev Rheumatol. 2012;8(8):444–5.

Aringer M, Günther C, Lee-Kirsch MA. Clin Immunol. 2012 Dec 12. doi:pii: S1521-6616(12)00291-4. 10.1016/j.clim.2012.11.012. [Epub ahead of print]

Atkinson JP, Yu CY. Systemic Lupus Erythematosus. SLE in Children. In: Robert G. Lahita, editors. 5th ed. Amsterdam: Elsevier Academic, 2011;21–45. Print.

Botto M. C1q knock-out mice for the study of complement deficiency in autoimmune disease. Exp Clin Immunogenet. 1998;15(4):231–4.

Brunner HI, Huggins J, Klein-Gitelman MS. Pediatric SLE–towards a comprehensive management plan. Nat Rev Rheumatol. 2011;7(4):225–33.

Crispín JC, Liossis SN, Kis-Toth K, Lieberman LA, Kyttaris VC, Juang YT, Tsokos GC. Pathogenesis of human systemic lupus erythematosus: recent advances. Trends Mol Med. 2010;16(2):47–57.

Flierman R, Daha MR. The clearance of apoptotic cells by complement. Immunobiology. 2007;212(4–5):363–70.

Gaubitz M, Schotte H. Early diagnosis in patients with systemic lupus erythematosus (SLE). Z Rheumatol. 2005;64(8):547–52.

Haas JP. Genetic background of juvenile idiopathic arthritis. Z Rheumatol. 2010;69(6):488–95.

Hauck F, Lee-Kirsch MA, Aust D, Roesler J, Pessler F. Complement C2 deficiency disarranging innate and adaptive humoral immune responses in a pediatric patient: treatment with rituximab. Arthritis Care Res (Hoboken). 2011;63(3):454–9.

Hedrich CM, Tsokos GC. Epigenetic mechanisms in systemic lupus erythematosus and other autoimmune diseases. Trends Mol Med. 2011;17(12):714–24.
Hedrich CM, Fiebig B, Hauck FH, Sallmann S, Hahn G, Pfeiffer C, Heubner G, Lee-Kirsch MA, Gahr M. Chilblain lupus erythematosus–a review of literature. Clin Rheumatol. 2008;27(8):949–54.
Hedrich CM, Zappel H, Straub S, Laass MW, Wieczorek K, Hahn G, Heubner G, Gahr M. Early onset systemic lupus erythematosus: differential diagnoses, clinical presentation, and treatment options. Clin Rheumatol. 2011;30(2):275–83.
Hersh AO, Trupin L, Yazdany J, Panopalis P, Julian L, Katz P, Criswell LA, Yelin E. Childhood-onset disease as a predictor of mortality in an adult cohort of patients with systemic lupus erythematosus. Arthritis Care Res (Hoboken). 2010;62(8):1152–9.
Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.
Lee-Kirsch MA, Chowdhury D, Harvey S, Gong M, Senenko L, Engel K, Pfeiffer C, Hollis T, Gahr M, Perrino FW, Lieberman J, Hubner N. A mutation in TREX1 that impairs susceptibility to granzyme A-mediated cell death underlies familial chilblain lupus. J Mol Med (Berl). 2007a;85(5):531–7.
Lee-Kirsch MA, Gong M, Chowdhury D, Senenko L, Engel K, Lee YA, de Silva U, Bailey SL, Witte T, Vyse TJ, Kere J, Pfeiffer C, Harvey S, Wong A, Koskenmies S, Hummel O, Rohde K, Schmidt RE, Dominiczak AF, Gahr M, Hollis T, Perrino FW, Lieberman J, Hübner N. Mutations in the gene encoding the 3′–5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus. Nat Genet. 2007b;39(9):1065–7.
Livingston B, Bonner A, Pope J. Differences in clinical manifestations between childhood-onset lupus and adult-onset lupus: a meta-analysis. Lupus. 2011;20(13):1345–55.
Livingston B, Bonner A, Pope J. Semin Arthritis Rheum. 2012 Dec;42(3):271-80. doi: 10.1016/j.semarthrit.2012.05.001. Epub 2012 Jun 15.
Mina R, Brunner HI. Systemic Lupus Erythematosus. SLE in Children. In: Robert G. Lahita, editors. 5th ed. Amsterdam: Elsevier Academic, 2011;573–97. Print.
Namjou B, Kothari PH, Kelly JA, Glenn SB, Ojwang JO, Adler A, Alarcón-Riquelme ME, Gallant CJ, Boackle SA, Criswell LA, Kimberly RP, Brown E, Edberg J, Stevens AM, Jacob CO, Tsao BP, Gilkeson GS, Kamen DL, Merrill JT, Petri M, Goldman RR, Vila LM, Anaya JM, Niewold TB, Martin J, Pons-Estel BA, Sabio JM, Callejas JL, Vyse TJ, Bae SC, Perrino FW, Freedman BI, Scofield RH, Moser KL, Gaffney PM, James JA, Langefeld CD, Kaufman KM, Harley JB, Atkinson JP. Evaluation of the TREX1 gene in a large multi-ancestral lupus cohort. Genes Immun. 2011;12(4):270–9.
Silverman E, Eddy A. Systemic Lupus Erythematosus. *Textbook of Pediatric Rheumatology*. James T. Cassidy, editors. 6th ed. Philadelphia, PA: Saunders, 2011;315–43. Print.
Truedsson L, Bengtsson AA, Sturfelt G. Complement deficiencies and systemic lupus erythematosus. Autoimmunity. 2007;40(8):560–6.
Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365(22):2110–21.
Zulian F, Pluchinotta F, Martini G, Da Dalt L, Zacchello G. Severe clinical course of systemic lupus erythematosus in the first year of life. Lupus. 2008;17(9):780–6.

Juvenile Idiopathic Arthritis

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Definition

Juvenile idiopathic arthritis (JIA) is defined as any arthritis of unknown origin that begins before the age of 16 years and persists for more than 6 weeks.

Introduction

JIA is not a single disease, but a diagnosis of exclusion that gathers together all forms of chronic arthritis of unknown origin that occurs in children and adolescents.

Historical Background

Different classification criteria have been used in the past in order to identify discrete clinical subsets that could correspond to different diseases. The more recent classification is the one proposed by the International League of

Associations for Rheumatology (ILAR) (Petty et al. 2004). The goal of this classification was to solve the problem of the previous heterogeneity in nomenclature and criteria between Europe and North America. The term JIA was adopted instead of those of juvenile chronic arthritis (JCA) or juvenile rheumatoid arthritis (JRA) previously in use in Europe and North America, respectively.

Epidemiology/Prevalence

Studies in Western populations have reported an incidence and a prevalence varying from 2 to 20 and from 16 to 150 per 100,000, respectively (Ravelli and Martini 2007).

The ILAR Classification

Seven disease categories were recognized, based on the features presented during the first 6 months of illness (Ravelli and Martini 2007; Cassidy et al. 2011; Prakken et al. 2011). However, some aspects of this classification have been recently challenged and suggestions for reconsidering classification and nomenclature have been proposed (Martini 2012).

Systemic Arthritis

It is considered the equivalent of "adult-onset Still's disease" and accounts for 10–15 % of JIA. It is characterized by prominent systemic features, such as high spiking fever, an evanescent, erythematous rash, hepatomegaly or splenomegaly, generalized lymphadenopathy, and serositis. Arthritis may be absent at onset and develop during the course of the disease. Laboratory investigation demonstrates leukocytosis (with neutrophilia), thrombocytosis, a high erythrocyte sedimentation rate (ESR) and C-reactive protein concentration, and marked elevations in serum ferritin levels. A microcytic anemia is also common.

The outcome is variable. In about half of patients, the disease is mainly characterized by the systemic features while the arthritis usually remits when systemic features are controlled. In the other half of patients, the disease follows an unremitting course; systemic symptoms may eventually resolve, leaving chronic arthritis as the major long-term problem.

About 5–8 % of children with systemic JIA develop a life-threatening complication, named macrophage activation syndrome (MAS). It is a form of reactive hemophagocytic lymphohistiocytosis and is characterized by sustained fever, pancytopenia, hepatosplenomegaly, liver insufficiency, coagulopathy, and neurological symptoms. Laboratory features include elevated triglycerides, and markedly increased ferritin concentrations. The demonstration of active phagocytosis of hematopoietic cells by macrophage in the bone marrow is common. Early recognition and treatment of MAS is essential.

Rheumatoid Factor Positive Polyarthritis

It is of rare occurrence (5 % of patients with JIA) and represents the childhood equivalent of adult rheumatoid factor (RF) positive rheumatoid arthritis; in fact, it is the only form of JIA with positive antibodies to cyclic citrullinated peptides.

Enthesitis-Related Arthritis

This is a form of undifferentiated spondyloarthritis characterized by the presence of enthesitis and arthritis. Most patients are HLA-B27 positive. The disease can be mild and remitting but a variable proportion of patients, who are impossible to identify at disease onset, develops the involvement of sacroiliac joints during disease course. All the different forms of adult spondyloarthritis (e.g., psoriatic arthritis, reactive arthritis, enteropathic arthritis, axial spondyloarthritis) may develop in children; the major difference in children is the much higher proportion of the undifferentiated forms.

Oligoarthritis

This is defined by inflammation of four or fewer joints during the first 6 months of disease. In Western countries, the large majority of patients belongs to a quite well-defined disease entity observed only in children and characterized by

an asymmetric arthritis, involving mainly large joints, an early onset (before 6 years of age), a female predilection, a high frequency of positive antinuclear antibodies (ANA), a high risk of developing chronic iridocyclitis, and consistent HLA associations.

Arthritis can remain limited to four or fewer joints (oligoarticular persistent) or can extend to affect five or more joints after the first 6 months of disease (oligoarticular extended); this second group of patients has a more guarded long-term prognosis.

Iridocyclitis may cause severe visual impairment and affects about 30 % of patients. The onset is insidious and is often entirely asymptomatic in contrast with the painful, acute uveitis that can be observed in spondyloarthritis. ANA positivity represents a strong risk factor for developing uveitis. Since uveitis is asymptomatic, children with ANA-positive JIA should be screened at least every 3 months by slit-lamp examination.

Rheumatoid Factor Negative Polyarthritis

This subset of JIA is defined by the involvement of five or more joints during the first 6 months of disease and is heterogeneous. At least two separate subsets can be identified: (a) one, characterized by a symmetric synovitis of large and small joints, onset in school age, and negative ANA, is similar to adult-onset RF-negative rheumatoid arthritis; (b) the second is similar in every respect to the above-mentioned early-onset, ANA-positive oligoarthritis, except for the number of joints affected in the first 6 months of disease. The similarities between this second subset and early-onset oligoarthritis strongly suggest that they represent different expressions of the same disease.

Psoriatic Arthritis

This is another heterogeneous category. If Vancouver criteria (presence of arthritis and psoriasis or some psoriatic features) are used to define psoriatic arthritis, two populations of patients are identified: (a) one that represents, as adult psoriatic arthritis, a form of spondyloarthropathy; (b) a second form that shares the same above-mentioned characteristics of ANA-positive, early-onset oligoarthritis. So it appears that the association of psoriasis with arthritis leads to the identification of two different subsets of patients, one that is similar to adult psoriatic arthritis and the other that overlaps with ANA-positive, early-onset oligoarthritis. The ILAR classification criteria for psoriatic arthritis, in which patients with enthesitis are by definition excluded, limit the identification of those patients that have a form of psoriatic arthritis similar to that observed in adults.

Undifferentiated Arthritis

This subset of JIA does not represent a separate subset, but a category which includes, by definition, patients that do not fulfill inclusion criteria for any other category, or are excluded by fulfilling criteria for more than one category.

Pathogenesis

As mentioned above, most of the forms of chronic arthritis in children represent the childhood counterpart of diseases also observed in adults. This is not the case for early-onset, ANA-positive oligoarthritis which is observed only in children; on the other hand, systemic JIA is much more common in childhood than adult-onset Still's disease and has therefore been studied mostly in children. Thus, in order to avoid overlaps with other entities only those immunological aspects that belong to systemic JIA and to early-onset ANA-positive arthritis will be discussed. For the other diseases, there are no relevant, specific immunological findings characteristic of childhood-onset forms.

Systemic JIA

Systemic JIA sets well apart from all the other forms of JIA (De Benedetti and Martini 1998; Mellins et al. 2011; Prakken et al. 2011). The systemic features, the marked inflammatory response, lack of sex bias, peak age at onset, lack of association with autoantibodies or HLA

antigens are all consistent with what is observed in autoinflammatory diseases.

Consistent with the supposed autoinflammatory nature of sJIA, gene expression studies on peripheral blood mononuclear cells (PBMCs) have shown an upregulation of genes associated with the activation of monocyte/macrophage lineage and a downregulation of the gene networks involving NK cells, T-cells, and MHC antigen-related biological processes. Two pro-inflammatory cytokines, interleukin (IL)-6, and IL-1, appear to play a major pathogenic role.

Circulating levels of IL-6 are markedly elevated, increase during the peak of fever, and correlate with the extent and severity of joint involvement. Synovial fluid levels of IL-6 are also significantly higher than those observed in other form of childhood or adult arthritis. The marked microcytic anemia is different from that observed in rheumatoid arthritis and is consistent with the effect of IL-6 on erythropoiesis. High circulating levels of IL-6 have been shown to cause growth failure, another feature of the disease. The important role of IL-6 has been confirmed more recently by the marked therapeutic effect of tocilizumab, a monoclonal antibody against the IL-6 soluble receptor (sIL-6R).

Evidence for the important role of IL-1 in the pathogenesis of sJIA came from the serendipitous finding of the marked therapeutic efficacy of anakinra, an IL-1 inhibitor which is the recombinant version of the naturally occurring soluble IL-1 receptor antagonist (IL-1 Ra). The role of IL-1 is not in contrast with that of IL-6 since IL-1 is upstream to IL-6. Of note, it has been shown that the response to anakinra can identity two different populations of patients. One (accounting for about 40 % of patients) shows a dramatic response to IL-1 blockade, similar to that observed in cryopyrin-associated autoinflammatory syndromes, leading to complete normalization of clinical as well as laboratory features in a few days. The other is resistant to treatment or shows an intermediate response. The main difference observed between these two groups was the number of active joints; patients with fewer joints affected had a higher probability of responding to anti-IL-1 therapy. The group with a more dramatic response could represent a separate entity in which the autoinflammatory component has the leading pathogenic role while autoimmune mechanisms may have also a more relevant role for the group with more joints involved.

It remains a mystery why sJIA is so strongly associated with macrophage activation syndrome (MAS). Of note, MAS is seldom observed in autoinflammatory diseases. As mentioned above, MAS bears a strong resemblance to a group of histiocytic disorders collectively known as hemophagocytic lymphohistiocytosis (HLH). The etiopathogenesis of MAS remains unknown. Immunologic abnormalities that are similar to those seen in HLH (poor NK cell cytolytic activity often associated with abnormal levels of perforin expression) have been reported, although these abnormalities may be reversible when associated with sJIA. However, in many instances of sJIA/MAS, no such defects in cytotoxic cell function have been identified. This suggests that MAS represents an end-stage clinical syndrome that can be elicited by different mechanisms.

Oligoarthritis

The homogeneity of this form of arthritis, characterized by an early onset and by ANA positivity, is witnessed by consistent associations with HLA antigens. Positive associations include HLA-A2, HLA-DRB1*11 (a subtype of HLA-DR5), and HLA-DRB1*08. On the contrary, HLA-DRB1*04 and HLA-DRB1*07 have been found to be significantly decreased (Ravelli and Martini 2007). HLA associations support an autoimmune pathogenesis and the early onset suggests that the disease could be elicited by a common infectious agent that could be encountered early in life. Of note, Barnes and coworkers (Barnes et al. 2010), studying gene expression, found that patients with early-onset arthritis ($\leq$6 years) had a characteristic B-cell signature, independent of the number of joints involved. An expansion of

activated switch memory B-cells and of IgG-secreting plasma blasts has been found in the SF of oligoarticular JIA patients (Corcione et al. 2009). Memory B-cells belonged to either the CD27+ or the CD27− subsets and expressed CD86, suggesting their involvement in antigen presentation to T-cells (Corcione et al. 2009). Another study (Morbach et al. 2011) has also confirmed that activated immunoglobulin class–switched CD27+ and CD27− memory B-cells – accumulate in the joints, further suggesting that B-cells play an antibody-independent immunopathologic role in oligoarthritis.

Synovial fluid Th17 cells have been showed to switch from a Th17 phenotype to a mixed Th1/Th17 phenotype, and, then, to a Th1 phenotype and that this switch was linked to the presence of IL-12 in the synovial fluid (Cosmi et al. 2011; Nistala et al. 2010). Moreover, within the joint, an inverse relationship between IL-17+ T-cells and FoxP3+ T-regulatory (Treg) cells has been found (Nistala et al. 2008). In JIA, Tregs are fully functional; nevertheless, Treg-mediated suppression of effector cells from the site of inflammation has been shown to be severely impaired. This resistance to suppression has been shown to be secondary to the activation of protein kinase B (PKB)/c-akt in inflammatory effector cells (Wehrens et al. 2011).

The fact that joint involvement can remain limited to 4 or less joints (oligoarticular persistent) or can extend to affect five or more joints after the first 6 months of disease (oligoarticular extended) provides an opportunity to study factors that are associated with disease extension. IL-17+ T-cell numbers were found to be higher and the ratio between SF regulatory and activated effector CD4 cells was found to be lower in patients with extended oligoarthritis as compared with patients with persistent oligoarthritis (Ruprecht et al. 2005; Nistala et al. 2008). Synovial CCL5 levels were found to be higher and SF CD4:CD8 ratio to be lower in children whose disease extended to a more severe phenotype during disease follow-up (Hunter et al. 2010). Gene expression profiling revealed increased levels of genes associated with inflammation and macrophage differentiation in patients with extended disease and of genes associated with immune regulation in patients with persistent oligoarticular disease (Hunter et al. 2010). CD4 expression and B-cell infiltrates are significantly higher and vascularization more pronounced in patients in whom arthritis extended to involve more joints with respect to those with persistent oligoarthritis (Finnegan et al. 2011).

The fact that persistent oligoarticular JIA is self-limiting and in about half of cases even self-remitting suggests that the disease may be responsive to endogenous regulation. Heat shock proteins (HSPs) are endogenous proteins that are expressed upon cellular stress, are able to modulate immune responses, and are ubiquitous at sites of inflammation, including the inflamed joints of JIA patients. Studies of patients with JIA have demonstrated the presence of antigen-specific T-cells directed against peptides derived from two types of HSPs, HSP60 and DnaJ (Prakken and Albani 2009). T-cell recognition of some peptides was associated with a regulatory functional phenotype. These responses were significantly augmented in patients with persistent oligoarticular JIA, suggesting a direct role in modulation of autoimmune inflammation. The identification of HSP tolerogenic peptides could have therapeutic implications in the future.

Management

With the exception of RF-positive polyarthritis, it is difficult to predict outcome at disease onset. Treatment (Ravelli and Martini 2007; Cassidy et al. 2011; Prakken et al. 2011) should therefore be tailored during disease course according to disease severity. Patients are initially treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and intra-articular corticosteroid injections (e.g., with triamcinolone hexacetonide) which have played an important role in preventing deformities secondary to joint contractures. In children who do not respond to

this approach (especially those with polyarthritis), methotrexate (MTX) has been shown to be effective in randomized trials. Although a trial has shown the efficacy of leflunomide in polyarticular JIA, the published experience with this drug in children is still limited. Patients who do not have an adequate response to MTX are often treated with biological agents usually added to MTX therapy. As in adult RA, the marked efficacy of anti-TNF agents and abatacept has been demonstrated in controlled trials of JIA while a phase III controlled trial with tocilizumab has just been completed.

Systemic JIA is initially treated with NSAIDs, but in most patients, corticosteroid therapy (e.g., prednisolone) is required. Patients who are steroid dependent often respond well to anti-IL-6 (tocilizumab) or to anti-IL-1 (e.g., anakinra or canakinumab) therapies. Response to MTX and anti-TNF agents is less dramatic in sJIA than that observed in the other JIA categories. The treatment of sJIA-associated MAS often relies on high-dose steroids and cyclosporine.

In iridocyclitis, early diagnosis is important for the success of therapy. The initial approach consists of glucocorticoid eye drops with mydriatics. For patients with resistant disease, systemic steroids may be required. If disease remains controlled, MTX, cyclosporine, monoclonal antibodies against TNF, and abatacept have been anecdotally reported to be effective.

Conclusions

JIA is not a single disease but an umbrella term that gathers together all forms of chronic arthritis with onset before 16 year of age. These forms appear to represent the childhood equivalent of adult disease with the exception of early-onset, ANA-positive arthritis which is a disease observed only in children. The other major difference between childhood and adult disease is the much higher frequency of systemic JIA compared with adult-onset Still's disease.

The last decade has witnessed a dramatic improvement in JIA treatment, thanks to the use of biological agents. TNF-blocking agents as well as abatacept have been shown to be effective in a high proportion of patients with polyarticular JIA. Systemic JIA has a peculiar immunological profile with marked involvement of innate immunity and responds well to IL-6 and to IL-1 inhibition (De Benedetti et al. 2012; Ruperto et al. 2012).

References

Barnes MG, Grom AA, Thompson SD, Griffin TA, Luyrink LK, Colbert RA, Glass DN. Biologic similarities based on age at onset in oligoarticular and polyarticular subtypes of juvenile idiopathic arthritis. Arthritis Rheum. 2010;62:3249–58.

Cassidy JT, Petty RE, Laxer RM, Lindsley CB, editors. Textbook of pediatric rheumatology. Philadelphia: Saunders Elsevier; 2011.

Corcione A, Ferlito F, Gattorno M, Gregorio A, Pistorio A, Gastaldi R, Gambini C, Martini A, Traggiai E, Pistoia V. Phenotypic and functional characterization of switch memory B cells from patients with oligoarticular juvenile idiopathic arthritis. Arthritis Res Ther. 2009;11(5):R150.

Cosmi L, Cimaz R, Maggi L, Santarlasci V, Capone M, Borriello F, Frosali F, Querci V, Simonini G, Barra G, Piccinni MP, Liotta F, De Palma R, Maggi E, Romagnani S, Annunziato F. Evidence of the transient nature of the Th17 phenotype of CD4+CD161+ T cells in the synovial fluid of patients with juvenile idiopathic arthritis. Arthritis Rheum. 2011;63:2504–15.

De Benedetti F, Brunner HI, Ruperto N, Kenwright A, Wright S, Calvo I, Cuttica R, Ravelli A, Schneider R, Woo P, Wouters C, Xavier R, Zemel L, Baildam E, Burgos-Vargas R, Dolezalova P, Garay SM, Merino R, Joos R, Grom A, Wulffraat N, Zuber Z, Zulian F, Lovell D, Martini A. Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis. N Engl J Med. 2012;367:2385–95.

De Benedetti F, Martini A. Is systemic juvenile rheumatoid arthritis an interleukin 6 mediated disease? J Rheumatol. 1998;25:203–7.

Finnegan S, Clarke S, Gibson D, McAllister C, Rooney M. Synovial membrane immunohistology in early untreated juvenile idiopathic arthritis: differences between clinical subgroups. Ann Rheum Dis. 2011;70:1842–50.

Hunter PJ, Nistala K, Jina N, Eddaoudi A, Thomson W, Hubank M, Wedderburn LR. Biologic predictors of extension of oligoarticular juvenile idiopathic arthritis as determined from synovial fluid cellular composition and gene expression. Arthritis Rheum. 2010;62:896–907.

Martini A. It is time to rethink juvenile idiopathic arthritis classification and nomenclature. Ann Rheum Dis. 2012;71:1437–9.
Mellins ED, Macaubas C, Grom AA. Pathogenesis of systemic juvenile idiopathic arthritis: some answers, more questions. Nat Rev Rheumatol. 2011;7:416–26.
Morbach H, Wiegering V, Richl P, Schwarz T, Suffa N, Eichhorn EM, Eyrich M, Girschick HJ. Activated memory B cells may function as antigen-presenting cells in the joints of children with juvenile idiopathic arthritis. Arthritis Rheum. 2011;63:3458–66.
Nistala K, Moncrieffe H, Newton KR, Varsani H, Hunter P, Wedderburn LR. Interleukin-17-producing T cells are enriched in the joints of children with arthritis, but have a reciprocal relationship to regulatory T cell numbers. Arthritis Rheum. 2008;58:875–87.
Nistala K, Adams S, Cambrook H, Ursu S, Olivito B, de Jager W, Evans JG, Cimaz R, Bajaj-Elliott M, Wedderburn LR. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. Proc Natl Acad Sci U S A. 2010;107:14751–6.
Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, He X, Maldonado-Cocco J, Orozco-Alcala J, Prieur AM, Suarez-Almazor ME, Woo P. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol. 2004;31:390–2.
Prakken BJ, Albani S. Using biology of disease to understand and guide therapy of JIA. Best Pract Res Clin Rheumatol. 2009;23:599–608.
Prakken B, Albani S, Martini A. Juvenile idiopathic arthritis. Lancet. 2011;377:2138–49.
Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet. 2007;369:767–78.
Ruperto N, Brunner HI, Quartier P, Constantin T, Wulffraat N, Horneff G, Brik R, McCann L, Kasapcopur O, Rutkowska-Sak L, Schneider R, Berkun Y, Calvo I, Erguven M, Goffin L, Hofer M, Kallinich T, Oliveira SK, Uziel Y, Viola S, Nistala K, Wouters C, Cimaz R, Ferrandiz MA, Flato B, Gamir ML, Kone-Paut I, Grom A, Magnusson B, Ozen S, Sztajnbok F, Lheritier K, Abrams K, Kim D, Martini A, Lovell DJ. Two randomized trials of canakinumab in systemic juvenile idiopathic arthritis. N Engl J Med. 2012;367:2396–406.
Ruprecht CR, Gattorno M, Ferlito F, Gregorio A, Martini A, Lanzavecchia A, Sallusto F. Coexpression of CD25 and CD27 identifies FoxP3+ regulatory T cells in inflamed synovia. J Exp Med. 2005;201:1793–803.
Wehrens EJ, Mijnheer G, Duurland CL, Klein M, Meerding J, van Loosdregt J, de Jager W, Sawitzki B, Coffer PJ, Vastert B, Prakken BJ, van Wijk F. Functional human regulatory T cells fail to control autoimmune inflammation due to PKB/c-akt hyperactivation in effector cells. Blood. 2011;118:3538–48.

Juvenile Idiopathic Arthritis: Pathogenesis, Presentation, and Treatment

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Synonyms

Juvenile chronic polyarthritis; Juvenile rheumatoid arthritis

Definition

Juvenile idiopathic arthritis (JIA) is a group of chronic arthritides with different presentations, disease courses, and outcomes. It is defined by the presence of inflammatory arthritis in children less than 16 years of age and lasting for at least 6 weeks duration. The International League of Associations for Rheumatology (ILAR) has classified JIA into seven subtypes based on prominent clinical and lab features. This classification system was revised in 2001 (Petty et al. 2004).

Historical Background

JIA is the most common chronic rheumatic disease in children and is an important cause of short- and long-term disability. In 1864, Cornil first described a young adult who developed inflammatory polyarthritis in her early teen years. Dr. Still described a classic case of chronic childhood arthritis in 1897. The terms "juvenile rheumatoid arthritis" and "juvenile chronic arthritis" were used for many years in the

United States (USA) and European countries, respectively, until a common terminology was introduced by ILAR in 1993 (Cassidy et al. 2011).

Epidemiology

One in every 1,000 children worldwide has JIA (Huang 2012). The incidence in the USA ranges from 9.2 to 13.9 per 100,000. The incidence is comparable to that in European countries.

JIA presents in children less than 16 years of age, but most commonly is seen between 1 and 6 years of age. Girls are affected twice as often as boys, but sex distribution varies by disease subtype.

Etiology and Pathogenesis

Infection has long been suspected to play a major role in the initiation and augmentation of JIA, especially parvovirus B19, Epstein-Barr virus, *Mycoplasma*, and *Streptococcus* (Cassidy et al. 2011; Gilliam et al. 2009; Reed et al. 2009). Molecular mimicry may be the mechanism by which these organisms lead to an increased risk of JIA.

Abnormalities in the adaptive or innate immune system leading to auto-reactive T-cell and autoantibody production in a genetically susceptible individual may lead to JIA (Lin et al. 2011). HLA genes especially A2, DRB1*04, DRB1*07 have been associated with oligoarticular JIA. HLA-DR4 has been associated with polyarticular JIA and HLAB27 with enthesitis-related arthritis.

There may be an imbalance of Th1/Th17 and Tregulatory (Treg) cells in favor of increased inflammatory cytokine production such as interferon-γ, IL-17, and IL-22. There is a decrease in the number and function of Treg cells, which subdue inflammation, because of Foxp3 deficiency.

Cytokines, especially tumor necrosis factor α (TNF-α) and IL-1 and IL-6, cause significant chronic inflammation, leading to tissue destruction. There is increased CD4+ T-cells and clonal expansion in the synovial fluid of JIA patients.

Presentation and Classification

As per ILAR classification, the different subtypes of JIA are noted in Table 1.

Systemic Arthritis (Systemic Onset Juvenile Idiopathic Arthritis (SoJIA))

SoJIA makes up 10 % of the JIA population. Though this form may present at any age, most often children are less than 5 years of age (Behrens et al. 2008). It is characterized by daily or twice daily high-grade fevers, temperatures exceeding 103 °F, known as quotidian fevers, which last for at least 2 weeks. These fevers are accompanied by an evanescent salmon colored rash, which may become more prominent during fever spikes, lymphadenopathy, hepatosplenomegaly, or serositis. Joint involvement may be delayed or initially may only present as migratory joint pain (Cimaz et al. 2012). The number of joints involved also varies. The anti-nuclear antibody (ANA) may be positive in 5–10 % of the patients, but rheumatoid factor (RF) is rarely seen in this subgroup. This form of JIA may mimic severe localized infections, sepsis, or malignancy, and thus, there may be delay in diagnosis and treatment. These patients are at risk of developing macrophage activation syndrome (MAS), a potentially life-threatening complication due to activation of T-cells and macrophages.

Oligoarthritis

Oligoarthritis is arthritis affecting one to four joints. It is the most common subtype of JIA,

Juvenile Idiopathic Arthritis: Pathogenesis, Presentation, and Treatment, Table 1 ILAR classification of JIA

Systemic arthritis
Oligoarthritis:
Persistent
Extended
Polyarthritis (rheumatoid factor negative)
Polyarthritis (rheumatoid factor positive)
Psoriatic arthritis
Enthesitis-related arthritis
Undifferentiated arthritis

accounting for 30–60 % of the JIA patients. It is more common in girls than boys and is seen in children younger than 4 years of age. Fifty percent of the time the child presents with monoarthritis with additional joints recruited later. This subtype is divided into persistent or extended. Persistent oligoarthritis affects four or fewer joints throughout the disease course. Extended oligoarthritis affects more than four joints after 6 months of disease onset. Uveitis, often "silent" or asymptomatic, accompanies this subtype of arthritis in 30 % of cases, especially those children with a positive ANA (Gowdie and Tse 2012). Ninety percent of JIA patients with uveitis have an elevated ANA titer (Moore 2010). Exclusions for this subtype of JIA are psoriasis or history of psoriasis in the patient or first-degree relative, arthritis in an HLA B27-positive male beginning after the sixth birthday, ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, reactive arthritis, acute anterior uveitis, or a history of one of these disorders in a first-degree relative (Petty et al. 2004).

Polyarthritis, Rheumatoid Factor Negative

By definition, this subgroup has more than four affected joints and RF is negative on serological testing. These patients have a variable disease onset (acute or insidious) and course (large or small joints involved). This subgroup, especially those with a positive ANA, may be hard to distinguish from ANA-positive extended oligoarticular JIA patients.

Polyarthritis, Rheumatoid Factor Positive

RF-positive children with polyarthritis have a clinical course similar to adult rheumatoid arthritis patients. These patients tend to be in their adolescence, and the arthritis tends to be aggressive, presenting with symmetric small joint involvement, especially of the hands and wrists. The anti-cyclic citrullinated peptide is often positive, the disease tends to be aggressive, and the prognosis is poor (Syed et al. 2008). This subgroup accounts for 5–10 % of all JIA patients. These patients may also develop rheumatoid nodules and are at higher risk for vasculitis.

Psoriatic Arthritis (Psoriatic Juvenile Idiopathic Arthritis, PsJIA)

This subgroup classically has arthritis and psoriasis. The clinical features include dactylitis (swelling of entire digits beyond the joint margin), nail changes (pitting: minimum of two, or onycholysis), or family history of first-degree relatives with psoriasis. The arthritis may precede the psoriasis by several years. RF tends to be negative. Patients with PsJIA are further divided into two groups. The first is those that appear similar to oligoarticular JIA, are younger, are more often female, and have an asymmetric oligoarticular presentation along with dactylitis. These children may also have asymptomatic anterior uveitis. The second group tends to be older, male, and have a higher incidence of spondyloarthritis (Gowdie and Tse 2012).

Enthesitis-Related Arthritis (ERA)

ERA is characterized by the presence of arthritis and/or enthesitis with at least two of the following: sacroiliac joint tenderness or inflammatory lumbosacral back pain, presence of HLA-B27 antigen, onset of arthritis in a male over 6 years of age, acute painful anterior uveitis (unlike oligoarticular JIA which tends to be silent), history of ankylosing spondylitis, sacroiliitis with inflammatory bowel disease, reactive arthritis, or acute anterior uveitis in a first-degree relative (Petty et al. 2004). More males than females tend to be affected by ERA. The arthritis tends to involve the lower extremity especially the hip joint and intertarsal joints of the feet. Entheses are sites of tendinous, ligamentous, or joint capsular insertions on bones. Typical entheses involved in ERA are in the lower extremities especially at the iliac crest, posterior and anterior superior iliac spine, femoral greater trochanter, ischial tuberosity, patella, tibial tuberosity, Achilles, and planter fascia. The axial skeleton is at risk of involvement in ERA. Some children will develop ankylosing spondylitis within 10–15 years of diagnosis. The HLA-B27 antigen is present in 80–90 % of these patients.

Undifferentiated Arthritis

JIA that fulfills criteria in none of the other categories listed above or fulfills two or more of the above categories is considered undifferentiated JIA.

Complications of JIA

Anterior uveitis is the most significant complication of JIA, mainly affecting the iris and ciliary body. It is insidious in onset and asymptomatic; thus, the recommendation is to refer all children diagnosed with JIA to an ophthalmologist for regular evaluation. Factors that increase the risk of uveitis are a positive ANA and age younger than 4 years. Females with oligoarticular JIA are at higher risk for uveitis than males. Children with a positive ANA should be seen every 3 months for a number of years and then every 6 months. All JIA patients should be monitored at least once a year for an indefinite period. Children with ERA mostly present with acute symptomatic uveitis.

Oligoarticular JIA patients are at risk for leg length discrepancy due to abnormal localized growth disturbance. Active inflammation leads to accelerated growth at the ossification center and premature epiphyseal closure of the affected limb, eventually leading to shortened limb on the involved side. The temporomandibular joint may also be affected, leading to micrognathia. Patients with severe active polyarticular JIA and SoJIA can have generalized linear growth disturbance because of circulating proinflammatory cytokines especially IL-6, which suppress growth at the growth plate as well as impair the production and function of insulin-like growth factor-1 (IGF-1) (Wong et al. 2008).

MAS, as mentioned earlier, is a complication mainly seen with SoJIA and is potentially life-threatening. MAS may be difficult to distinguish from a flare of SoJIA and is characterized with sustained fevers, hepatosplenomegaly, anemia, liver dysfunction, rashes, coagulopathy, decreasing white cell counts and platelets, decreasing ESR, increased ferritin level, and hemophagocytosis on bone marrow aspirate (Gowdie and Tse 2012).

Children with JIA are at risk for osteopenia and osteoporosis. Potential risk factors for abnormal bone density include chronic active and persistent inflammation, the chronic use of corticosteroids, decreased physical activity, and decreased sunlight exposure. Besides controlling the disease with medications, patients are encouraged to exercise and follow a nutritionally balanced diet. Vitamin D supplementation is important. Multiple studies have shown vitamin D enhances the innate immune system and helps dendritic cells differentiate and mature in response to antigen presentation and enhances the function of Treg cells. Vitamin D also downregulates proinflammatory cytokines such as IL-12 as well as Th17 cells (Huang 2012).

Treatment

Physical and occupational therapy are crucial parts for the treatment of JIA to preserve/improve joint function and gain muscle strength. Splints or braces are sometimes required to allow for better function and joint stabilization. Aquatic therapy is an excellent means of strengthening muscles and stabilizing joints without significant strain on joints.

The initial pharmacologic treatment of JIA is usually nonsteroidal anti-inflammatory agents (NSAIDs). Corticosteroids may be required for more severe and widespread disease or if there is systemic involvement such as serositis, fevers, rash, or lymphadenopathy as in the case of SoJIA or polyarticular JIA. The different classes of medications are listed in Table 2.

Recently, Beukelman and colleagues published the 2011 ACR JIA treatment guidelines based on consensus among pediatric rheumatologists and the review of the literature. This is a good resource for physicians caring for JIA patients (Ringwold et al. 2013).

Oligoarticular JIA will often respond to initial treatment with an NSAID. These agents work via inhibition of the cyclooxygenase pathway of arachidonic acid metabolism and prevention of proinflammatory prostaglandin production (Kahn 2011). The main side effect is abdominal pain or headaches.

Juvenile Idiopathic Arthritis: Pathogenesis, Presentation, and Treatment, Table 2 Medication classes used in JIA

Nonsteroidal anti-inflammatory (NSAIDs)
Naproxen[a]
Ibuprofen[a]
Meloxicam[a]
Tolmetin[a]
Diclofenac sodium
Nabumetone
Indomethacin[a]
Sulindac
Celecoxib[a]
Disease-modifying antirheumatic agents (DMARDs)
Methotrexate
Hydroxychloroquine
Sulfasalazine
Leflunomide
Corticosteroids
Prednisone
Prednisolone
Intra-articular triamcinolone injection
Intravenous methylprednisolone
Biologic DMARDs
Anti-tumor necrosis factor agents (anti-TNFs)
Etanercept
Adalimumab
Infliximab
IL-1 receptor antagonist – Anakinra
CTLA-4 costimulatory blockade – Abatacept
IL-6 receptor antibody – Tocilizumab

[a]NSAIDs FDA approved for children <18 years of age

If swelling persists in oligoarticular JIA after a trial of NSAIDs, often the caregiver will choose to inject corticosteroid, preferably triamcinolone hexacetonide in the affected joint to help resolve the swelling and avoid leg length discrepancy. Once the arthritis is noted to be in remission, the NSAID treatment may be continued for another 6 months, when the patient is reassessed. If the patient continues to show no signs of active disease, discontinuing the NSAIDs may be considered.

The oligoarticular extended JIA patients should be treated like polyarticular patients. In most cases, these patients will require DMARDs to bring their disease under control. Because these medications take some time to show their effects, systemic corticosteroids will often be recommended in low doses as bridging therapy. Methotrexate is the most commonly prescribed DMARD as multiple studies have confirmed its benefits in JIA and overall it is well tolerated in children (Kahn 2011). This medication is prescribed orally once weekly along with folic acid to minimize side effects. It is available in tablets (2.5 mg each) or liquid 25 mg/ml. The dosage in a pediatric patient is 10–20 mg/M^2. Since methotrexate can cause liver abnormalities, leucopenia, or thrombocytopenia, labs should be checked routinely every 6–8 weeks. Methotrexate can also rarely cause pneumonitis, manifested by a chronic dry cough. This is most commonly seen in the initial 6–12 weeks of exposure but may be seen any time in the treatment course.

Hydroxychloroquine (HCQ) is an antimalarial antibiotic, which exerts anti-inflammatory effects via stabilization of lysosomal membrane. It inhibits lysosomal function by impairing lysosomal acidification which, in turn, inhibits TLR-7 and -9 activation (Ventuturupalli et al. 2012). By inhibiting lysosomal function, it also causes cytokine inhibition, including IL-6. In JIA, it is not usually the first DMARD to be used, but is often added to methotrexate or other DMARDs to potentiate effects. HCQ is only available in tablet form and the recommended dosage is 6 mg/kg per day.

Sulfasalazine is less often used in the USA than in Europe. It may be used as monotherapy or in combination with other DMARDs. It is started at 50 mg/kg.

Leflunomide is a pyrimidine synthetase inhibitor, which has also been shown to be effective in children with JIA. It should be used with caution in female JIA patients because of its teratogenic effects and long half-life.

Biologic DMARD therapy is often used in juvenile arthritis refractory to conventional NSAIDs and DMARDs. In 1999, etanercept was the first biologic to receive FDA approval for polyarticular JIA (Lovell et al. 2000). It is a soluble fusion protein consisting of human p75 TNF receptor fused to the Fc region of human IgG1. It is available as a weekly subcutaneous injection at a dose of 0.8 mg/kg/week.

Its main side effects are infection risk, demyelinating disease, decreased capability to mount response to vaccine, and possibly malignancy, mainly lymphomas. In adult RA patients, an increased rate of *Mycobacterium tuberculosis* (TB) was noted in patients initiated on anti-TNF agents and thus, all patients should be screened for TB with skin testing and a chest x-ray prior to initiation and annually while on treatment.

Adalimumab is a humanized IgG monoclonal anti-TNF-α antibody, which was FDA approved for polyarticular JIA in 2008. It is also a subcutaneous injection available in a 20-mg dose (for children weighing 10–30 kg) or 40-mg dose (for children weighing >30 kg), injected once every 2 weeks. It has similar side effects as mentioned above for etanercept.

Infliximab is a chimeric (mouse-human) IgG1 monoclonal antibody administered intravenously. It binds to both membrane-bound as well as soluble TNF-α. Though it is not FDA approved for JIA, a 2007 multicenter, randomized, double-blind placebo-controlled trial in 122 polyarticular JIA demonstrated ACR Pedi 50 and 70 responses in 70 % and 52 % of the patients, respectively (Ruperto et al. 2007). Because of its chimeric nature, some patients form human anti-chimeric antibodies (HACAs) and deceased efficacy. It is dosed at 3–10 mg/kg every 6–8 weeks.

Anakinra is a recombinant IL-1 receptor antagonist available as daily subcutaneous injections. It is used for SoJIA patients because IL-1 is increased in this subtype. The systemic symptoms of fever, rash, and elevation of white cells, platelets, and other acute phase reactants tend to improve with Anakinra. It is dosed at 1–2 mg/kg/daily. There is a higher rate of injection site reaction with Anakinra than with anti-TNF agents.

Not all JIA patients will respond to anti-TNF therapy or IL-1 therapy, suggesting other cytokines may play a role in certain patients. Abatacept is another option; it is a soluble fully humanized fusion protein consisting of an extracellular domain of CTLA-4 linked to modified Fc portion of human IgG. It gained FDA approval for polyarticular JIA in 2008. It binds to CD80/CD86 on antigen-presenting cells, inhibiting binding of CD28 on T-cells and consequently inhibiting T-cell activation. It is administered at a dose of 10 mg/kg every 4 weeks after an initial loading regimen. Infections are the most concerning side effect for abatacept. Like anti-TNF agents, it may also increase the risk of malignancy. In adult patients with chronic obstructive pulmonary disease (COPD), abatacept may cause exacerbation of underlying lung disease.

Tocilizumab is a humanized monoclonal antibody directed against IL-6 receptor, which was FDA approved for SoJIA in 2011 and is undergoing trials for polyarticular JIA. It is intravenously administered at a dose of 12 mg/kg for children weighing <30 kg and 8 mg/kg for children weighing >30 kg every 2 weeks. Its side effects include infections, infusion reactions, blood dyscrasias (especially leucopenia), liver enzyme abnormalities, and hyperlipidemia.

Summary

JIA has many phenotypically different presentations classified by the ILAR into seven subtypes. If left untreated, it can cause leg length discrepancy, generalized stunted growth, micrognathia, uveitis, and systemic complications such as MAS, which has high mortality. The uveitis tends to be asymptomatic (thus termed "silent" uveitis). Children with oligoarthritis should have regular eye exams every 6 months and more often (every 3 months) for those JIA patients with a positive ANA.

Elevated levels of TNF, IL-1, IL-6, and other cytokines have been found in patients with JIA, and treatments have been targeted to these cytokines. The initial treatment for oligoarthritis and ERA is NSAID while corticosteroids and DMARDs (especially methotrexate) are used often in early polyarthritis and SoJIA. Biologic DMARDs are initiated in refractory cases in most onset types or as first line should a JIA patient have axial disease (sacroiliitis).

More trials are underway to better understand JIA and to design better treatments. Childhood Arthritis and Rheumatology Research

Alliance (CARRA) is currently working on a multicenter registry for children with rheumatic diseases. More than 60 pediatric rheumatology centers are involved in the registry and more than 7,000 children have already been enrolled.

Cross-References

- ▶ Autoinflammatory Diseases
- ▶ Genetics of Juvenile Idiopathic Arthritis

References

Behrens EM, Beukelman T, Gallo L, Spangler J, Rosenkranz M, Arkachaisri T, Ayala R, Groh B, Finkel TH, Cron RQ. Evaluation of the presentation of systemic onset juvenile rheumatoid arthritis: data from the Pennsylvania Systemic Onset Juvenile Arthritis Registry (PASOJAR). J Rheumatol. 2008;35(2):343–8.

Cassidy JT, Petty RE, Laxer R, Lindsley C. Textbook of pediatric rheumatology. 6th ed. Philadelphia: Saunders Elsevier; 2011.

Cimaz R, Scheven AV, Hofer M. Systemic-onset juvenile idiopathic arthritis: the changing life of a rare disease. Swiss Med Wkly. 2012;142:w13582.

Gilliam BE, Reed MR, Syed RH, Moore TL. Rheumatic manifestation of Epstein-Barr virus in children. J Pediatr Infect Dis. 2009;4:333–42.

Gowdie PJ, Tse SML. Juvenile idiopathic arthritis. Pediatr Clin N Am. 2012;59:301–27.

Huang JL. New advances in juvenile idiopathic arthritis. Chang Gung Med J. 2012;35:1–14.

Kahn P. Juvenile idiopathic arthritis: an update on pharmacology. Bull NYU Hosp Jt Dis. 2011;69(3):264–76.

Lin YT, Wang CT, Gershwin ME, Chiang BL. The pathogenesis of oligoarticular/polyarticular vs systemic juvenile idiopathic arthritis. Autoimmun Rev. 2011;10:482–9.

Lovell DJ, et al. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. N Engl J Med. 2000;342(11):763–9.

Moore TL. Juvenile idiopathic arthritis. In: Ravel RE, Bope ET, Kellerman R, editors. Conn's current therapy. Philadelphia: Saunders Elsevier;2010, pp 987–9.

Petty RE, Southwood TR, Manners P, Baum J, Galss DN, Goldenberg J, He X, Maldonado-Cocco J, Orozco-Alcala J, Prieur AM, Suarez-Almazor ME, Woo P, International League of Associations for Rheumatology. International League of Associations for rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol. 2004;31:390–2.

Reed MR, Gilliam BE, Syed RH, Moore TL. Rheumatic manifestation of Parvovirus B19 in children. J Pediatr Infect Dis. 2009;4:321–31.

Ringwold S et al. 2013 update of the 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: recommendations for the medical therapy of children with systemic juvenile idiopathic arthritis and tuberculosis screening among children receiving biologic medications. Arthritis Rheum. 2013;65(10):2499–512. doi:10.1002/art.38092.

Ruperto N, et al. A randomized, placebo-controlled trial of infliximab plus methotrexate for the treatment of polyarticular-course juvenile rheumatoid arthritis. Arthritis Rheum. 2007;56(9):3096–106.

Syed RH, Gilliam BE, Moore TL. Rheumatoid factors and anticyclic citrullinated peptide antibodies in pediatric rheumatology. Curr Rheumatol Rep. 2008; 10:156–63.

Ventuturupalli S, Gudsoorkar V, Wallace D. Reconsidering antimalarials in systemic lupus erythematosus: developments of translational clinical interest. J Rheumatol. 2012;39(9):1769–71.

Wong SC, MacRae VE, Gracie JA, McInnes IB, Galea P, Gardner-Medwin J, Ahmed SF. Inflammatory cytokines in juvenile idiopathic arthritis: effects on physical growth and the insulin-like-growth factor axis. Growth Horm IGF Res. 2008;18(5):369–78.

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Kidney Involvement in Idiopathic TTP/HUS

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Synonyms

Atypical HUS; Hemolytic uremic syndrome; HUS; Thrombotic microangiopathy; Thrombotic thrombocytopenic purpura; TMA; TTP

Definition

Discussing kidney involvement in thrombocytopenic purpura (TTP)/hemolytic uremic syndrome (HUS) is complicated because exactly what disease is identified by these diagnoses has been a moving target ever since 1925. This work focuses on the kidney involvement to that associated with *idiopathic* TTP/HUS. What is meant by this terminology and how we got to that point are important to the topic. It is discussed below.

Introduction

In 1925 Moschcowitz reported autopsy findings of widespread arteriolar hyalin thrombi in a 16-year-old girl who presented with unexplained fever and heart failure and then lapsed into coma and died (Moschcowitz 2003). By the 1930s, additional similar cases were recognized. These showed that the thrombi were composed mainly of platelets and that central nervous system involvement was more common than kidney involvement (Baehr et al. 1936). By the 1950s, further detail emerged, particularly that the affected generally were adults, a thrombotic microangiopathy was present (causing a microangiopathic hemolytic anemia), and the patients often were severely thrombocytopenic. By this time the diagnostic term "thrombotic thrombocytopenic purpura (TTP)" was accepted.

In the late 1950s, the field once again became unsettled when Glasser reported a group of patients with a thrombotic microangiopathy that matched that of TTP except that the affected were children, and severe kidney failure was the dominant clinical presentation. Because of its strong association with acute kidney injury (AKI), this condition was termed "hemolytic uremic syndrome" (HUS). During the ensuing years, TTP and HUS were generally regarded as separate entities. Indeed, by the mid-1980s, it became apparent that many cases of HUS could be attributed to infections, particularly E. coli 0157:H7. This form of HUS was later termed D-HUS

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(diarrhea-associated HUS). A further differentiation within the diagnosis of HUS emerged when it was discovered that genetic or acquired deficiency of regulators of the alternative pathway could also cause HUS. This condition, which is rare, was termed "atypical (a) HUS" to differentiate it from the "typical" HUS, which is associated with diarrhea (Noris and Remuzzi 2009; George 2010).

Despite these clarifications there remained a large number of patients who presented with microangiopathic hemolytic anemia and target organ injury in whom their condition was neither post-diarrheal nor associated with unregulated alternative complement pathway activation or any other known cause of thrombotic microangiopathy. From this emerged the notion that *idiopathic* TTP and *idiopathic* HUS were different aspects of the same disease (Remuzzi 1987). On this basis, it could be argued that the term "*idiopathic* HUS" is redundant when used in conjunction with *idiopathic* TTP. However, many experts discuss them together to ensure that they receive the same treatment. So, this work will continue the tradition of linking HUS to TTP, if the HUS is *idiopathic* (i.e., not "typical" HUS or "atypical HUS," as will be discussed later).

Diagnostic Approach to *Idiopathic* TTP/HUS

A potentially defining moment in differentiating *idiopathic* TTP/HUS from non-idiopathic HUS/TTP occurred in the late 1990s when it was determined that many patients with syndromes consistent with TTP or HUS had severely decreased activity of the plasma enzyme ADAMTS-13 (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats) (Tsai and Lian 1998; Furlan et al. 1998). This enzyme degrades the ultra large (UL) polymers of von Willebrand's factor (vWF) thereby mitigating their ability to induce platelet aggregation and the platelet thrombi that characterize TTP. Unfortunately, this remarkable advance still did not allow an absolute separation of *idiopathic* and non-idiopathic forms of TTP/HUS for the following reasons:

1. Not all patients with the clinical syndromes of *idiopathic* TTP/HUS have severe ADAMTS-13 deficiency (George 2010). Also, in rare instances, severe ADAMTS-13 deficiency may not be fully expressed until later in the patient's course. This may be for methodological reasons related to the assay (Froehlich-Zahnd et al. 2012).
2. Not all patients with severe ADAMTS-13 deficiency develop a thrombotic microangiopathy (George 2010).
3. Treatment aimed at restoring ADAMTS-13 levels towards normal by repeated plasma exchange using fresh frozen plasma is highly effective in reversing *idiopathic* TTP/HUS and preventing death, even in those who do not have severe ADAMTS-13 deficiency (Vesely et al. 2003).

Despite these gaps in understanding of the role of ADAMTS-13 in the pathogenesis of TTP and HUS, the diagnostic puzzle has begun to fit together much better, enough so that many experts in the field now regard *idiopathic* TTP and *idiopathic* HUS as variations of the same condition. These same experts (Wolf 2004; George 2010; Zheng et al. 2010; Lasik and Silva 2007) are comfortable in making a working diagnosis of *idiopathic* TTP/HUS on the basis of the following:

- The patient presents with evidence of a microangiopathic hemolytic anemia (Table 1).
- Target organ injury is present (e.g., central nervous system, kidney, heart, skin, and intestinal tract).
- The microangiopathic hemolytic anemia and target organ injury are not explained by *any other* condition that can mimic *idiopathic* TTP/HUS (Table 2).

Current expert opinion is that to make a working diagnosis of *idiopathic* TTP/HUS does not require documentation that ADAMTS-13 activity is severely decreased: <5 % of normal (Zheng et al. 2010) or <10 % of normal (George 2010). That documentation can occur later. The rationale is for both methodological reasons

Kidney Involvement in Idiopathic TTP/HUS, Table 1 Criteria for the diagnosis of microangiopathic hemolytic anemia[a]

Criterion	Comment
Anemia	The reticulocyte count eventually becomes elevated indicating that the anemia is not the result of low red cell production
Schistocytes on blood smear	Usually 2 % or more if red cells in the peripheral smear are schistocytes, although rarely they are absent (Brilliant et al. 1996; Daram et al. 2005)
Low plasma haptoglobin	This indicates that the anemia is from intravascular destruction of red cells rather than blood loss
Elevated serum LDH	This is evidence of red cell hemolysis and release of LDH from ischemic tissue (Cohen et al. 1998)
Thrombocytopenia[b]	This results from either the primary consumption of platelets (e.g., the platelet thrombi of *idiopathic* TTP/HUS) or from the secondary consumption of platelets in fibrin thrombi (e.g., catastrophic antiphospholipid syndrome)

[a]Microangiopathic hemolytic anemia is the hallmark of thrombotic microangiopathies such as TTP/HUS. However, microangiopathic hemolytic anemia can occur in disease states that generally are not classified as a thrombotic microangiopathy (see Table 2)

[b]The diagnostic usefulness of measuring high-sensitivity D-dimer in thrombocytopenic patients in order to differentiate those in whom the thrombocytopenia is the result of lack of platelet production, or platelet consumption in a thrombotic process, has not, to the best of our knowledge, been carefully studied. It is reported that fibrin degradation products are not elevated in TTP (George 2011) or they are elevated only in those with "tissue ischemia" (Leung 2010). However, the latter reference is from an era before high-sensitivity D-dimers were measured and, presumably, the assays were less sensitive. Also, the former reference does not describe how tissue ischemia was validated. It is plausible that those with elevated LDH had elevated D-dimers because they had a more extensive thrombotic arteriopathy. This, in turn, caused the tissue ischemia. So, elevated D-dimer may be a measure of the thrombotic process

(standardized assays for ADAMTS-13 are not widely available) and practical reasons (the assay may not be available in time to inform patient management). The latter is a crucial point because, if the patient does have acute *idiopathic* TTP/HUS, it is essential to begin treatment with fresh frozen plasma exchange as soon as feasible in order to protect the patient from the severe acute manifestations of *idiopathic* TTP/HUS (George 2010).

Although the above approach may seem straightforward, it is complicated for the following reasons:

1. There are a large number of other conditions that must be differentiated from the patient's condition before a diagnosis of *idiopathic* TTP/HUS can be made and plasma exchange is justified (Table 2).
2. The diagnostic and therapeutic approach to the patient must also take into account those conditions that are not *idiopathic* TTP/HUS but still may be benefitted by plasma exchange with fresh frozen plasma or 5 % albumin (Table 2).
3. The decision to deploy plasma exchange should not be made lightly because of the costs and risks of plasma exchange (Howard et al. 2006).

Although severe ADAMTS-13 deficiency is not required to establish a diagnosis of *idiopathic* TTP/HUS, it is widely believed that severe ADAMTS-13 deficiency (<5 % of normal) is highly sensitive and specific in differentiating *idiopathic* from non-idiopathic TTP/HUS (Bianchi et al. 2002; Raife et al. 2004; George 2010).

Pathogenesis of *Idiopathic* TTP/HUS

Severe deficiency of ADAMTS-13 activity may be either genetic or acquired. Acquired *idiopathic* TTP/HUS is usually caused by IgG autoantibodies to ADAMTS-13 and particularly the IgG1 and IgG4 subtypes. IgA and IgM isotypes have also been described (Zheng et al. 2010; Tsai 2010). If ELISA is used for detection, inhibitors are detectable in approximately 97 % of cases with severe ADAMTS-13 deficiency but in only 50–90 % of cases when functional assays are used (Zheng et al. 2010). These autoantibodies have also been found in the TTP/HUS associated with systemic autoimmune conditions such as SLE.

In patients with genetic deficiency of ADAMTS-13, homozygous or compound heterozygous mutations of ADAMTS-13 protease are found (Tsai 2010). This causes severe deficiency of ADAMTS-13 activity and causes TTP/HUS.

Kidney Involvement in Idiopathic TTP/HUS, Table 2 Conditions that mimic *idiopathic* TTP/HUS because they present with a microangiopathic hemolytic anemia and target organ injury. They are stratified according to whether they are benefitted by plasma exchange[a]

Conditions that present with microangiopathic hemolytic anemia and, like idiopathic TTP/HUS, may be benefited by plasma exchange	
Condition	Comment
1. Severe *primary* antiphospholipid syndrome	Systemic anticoagulation is also needed for acute and chronic management. High-dose steroid therapy is also recommended for initial management. Plasma exchange with fresh frozen plasma is recommended, but 5 % albumin may also be beneficial
2. Severe *secondary* antiphospholipid syndrome	Usually these patients have SLE. If the SLE is active, this must also be treated with steroids and immunosuppression
3. TTP/HUS associated with SLE	If the SLE is active, this must also be treated
4. ANCA-associated vasculitis	Anemia and schistocytes in the peripheral blood smear are not uncommon in this condition. Usually the anemia is mild. Plasma exchange with 5 % albumin is indicated if severe renal failure is present
5. Severe type 1, 2, or 3 cryoglobulinemia	Plasma exchange with 5 % albumin is acutely helpful. Some may have severe hypertension mimicking the microangiopathic hemolytic anemia of malignant hypertension (George 2010)
6. Congenital TTP	This is a rare genetic polymorphism of the ADAMTS-13 molecule. Plasma exchange can be used, although plasma infusion usually is sufficient (George 2010)
7. Atypical (a) HUS	This is a rare condition that may be benefited by plasma exchange with fresh frozen plasma until the diagnosis is established. After than eculizumab, a monoclonal antibody that targets C5 of the terminal complement pathway may be more effective
Conditions that present with microangiopathic hemolytic anemia, but plasma exchange has not been shown to be beneficial	
Condition	Comment
1. Typical HUS (diarrhea associated)	Resolution usually occurs with conservative care
2. Drugs:	
Cancer chemotherapy	Mitogenic C, bleomycin and cisplatin, anti-VEGF
Immunosuppressives	Cyclosporin, tacrolimus
Anti-VEGF	As cancer chemotherapy or as intraocular injections for macular degeneration
Quinine for muscle cramp	Quinine is also contained in beverages
Ticlopidine, clopidogrel	Ironically, these are antiplatelet drugs
Therapies associated with stem cell transplant	Multiple drugs may be involved
Other drugs	Oral contraceptives, valacyclovir
3. Malignant hypertension	This can be a difficult condition to separate from conditions that are benefited by plasma exchange because they are sometimes associated with acute severe hypertension
4. Severe preeclampsia, HELLP syndrome	Rarely these may actually be TTP/HUS triggered by pregnancy. So, plasma exchange may be indicated
5. Acute and subacute infection	Bacterial, fungal, parasitic, and viral infection (including HIV) may trigger microangiopathic hemolytic anemia (Booth et al. 2011; Blum et al. 2011). Antimicrobial therapy generally is beneficial
6. Scleroderma renal crisis	Malignant hypertension is usually present. Blood pressure control usually is beneficial
7. Active SLE in the absence of antiphospholipid syndrome, ADAMTS-13 deficiency, or demonstrable clot (venous, artery, or arteriole)	These patients have elevated D-dimers consistent with platelets being consumed in a thrombotic process. These patients recover with steroid and immunosuppressive therapy. Likely this condition is the result of immune complex-mediated endothelial injury that resolves with steroid and immunosuppressive therapy (Wu et al. 2008)
8. Disseminated malignancy	Mucin-producing tumor may be particularly involved

(*continued*)

Kidney Involvement in Idiopathic TTP/HUS, Table 2 (continued)

Conditions that present with microangiopathic hemolytic anemia, but plasma exchange has not been shown to be beneficial	
Condition	Comment
9. Atheroembolism	This can be acute or subacute embolism. It can closely mimic *idiopathic* TTP/HUS
10. Severe "bath salts" intoxication	Bath salts are illegal methamphetamines (unpublished personal observations)

[a]Recommended as a general reference is the current section in UpToDate titled "Causes of TTP/HUS in adults"

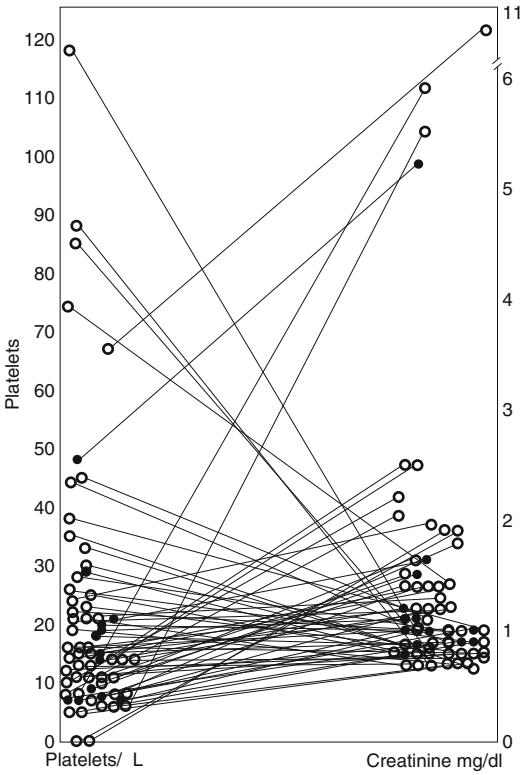
Heterozygotes for the ADAMTS-13 mutations usually have 40–70 % ADAMTS-13 activity and no TTP (Tsai 2010). Patient's homozygous or compound heterozygotes for mutations of ADAMTS-13 protease will manifest TTP.

Monitoring ADAMTS-13 activity level and inhibitor titers is important to long-term management because ADAMTS-13 levels that remain less than 5–10 % after treatment of TTP are associated with a high risk of relapse and poor long-term outcomes (Zheng et al. 2010; Tsai 2010; George 2011).

Kidney Involvement in *Idiopathic* TTP/HUS

Kidney Function. Our review of the literature with regard to *idiopathic* TTP/HUS did not reveal any reports in which all of the key manifestations of kidney injury (hematuria, proteinuria, decreased GFR) were described in detail in individual patients. However, mention is made in general reviews that proteinuria in this condition is moderate (e.g., 24-h proteinuria 1–2 g) and hematuria is usually present. There is, however, a report of individual serum creatinine values in 64 patients with *idiopathic* TTP/HUS and whose ADAMTS-13 level was <5 % of normal. This is displayed in Fig. 1 which shows that at presentation only about 25 % of the patients manifested serum creatinine values >1.3 mg/dl and that severe AKI was uncommon.

Figure 1 is also of interest because the serum creatinine values are shown in relationship to the platelet count in these individual patients, all of whom had severe ADAMTS-13 deficiency (<5 %, open circles) or less severe ADAMTS-13 deficiency (5–10 %, closed circles) (Vesely et al. 2003). As shown, there was no consistent relationship between the presence or absence of



Kidney Involvement in Idiopathic TTP/HUS, Fig. 1 Relationship between platelet count and serum creatinine at presentation in patients deemed to have an *idiopathic* TTP/HUS (Zheng et al. 2010). Each *line* joins the values for platelet count and serum creatinine level for a discrete patient. The data were adapted from Table 1 of Zheng XL (Zheng et al. 2010), with permission of the publisher. The *open circles* are those in which the ADAMTS-13 activity was <5 % of normal. The closed circles are those who had ADAMTS-13 levels that were 5–10 % of normal. As shown, there was no consistent relationship between the degree of thrombocytopenia and the degree of serum creatinine elevation at presentation. In addition, there was no consistent relationship between platelet count and serum creatinine according to whether the ADAMTS-13 activity was <5 % of normal or 5–10 % of normal (see text for further discussion)

severe ADAMTS-13 deficiency and platelet count or serum creatinine level. Figure 1 is further evidence that, although severe ADAMTS-13 deficiency may be highly sensitive and specific for *idiopathic* TTP/HUS, within that category it does not appear to distinguish between those presenting with or without impaired kidney function.

Kidney Biopsy Findings. The published data in this domain are even more limiting. Most of the reported kidney biopsy findings are from an era in which a distinction was made between *idiopathic* TTP and *idiopathic* HUS. This may explain some of the inconsistencies in the published data. For example, although it is stated that *idiopathic* TTP and *idiopathic* HUS can be regarded as variations on the same condition, the interpretation of the kidney biopsies in those deemed to have TTP was different from those deemed to have HUS. For example, the presence of arteriolar platelet thrombi is emphasized in those deemed to have TTP (Lasik and Silva 2007) whereas the presence of fibrin thrombi with platelets is emphasized in those deemed to have HUS (Lasik and Silva 2007). These comparisons likely are further confounded by bias in selecting the patients to be biopsied. The patients deemed to have TTP often had lower platelet counts and better kidney function than those deemed to have HUS. So, likely the "TTP" cohort was less likely to undergo kidney biopsy than the "HUS" patients. Given these limitations, we suggest the following is a reasonable summary of the published evidence regarding the kidney biopsy findings in *idiopathic* TTP/HUS (Lasik and Silva 2007):

(a) The light microscopy shows thrombi in glomeruli and arterioles. These represent patients with disease of recent onset. In patients with more chronic disease, there is conspicuous arteriolar change with intimal hyperplasia and marked narrowing of capillary lumens.
(b) Immunofluorescence microscopy shows that the thrombi are either mainly platelets or mainly fibrin thrombi.
(c) Electron microscopy shows glomerular subendothelial "fluffy" deposits that are thought to represent material derived from fibrin deposits or from cellular debris.

In summary, the kidney manifestations of *idiopathic* TTP/HUS have not been studied in detail and have not been studied with the view of differentiating *idiopathic* HUS/TTP from non-idiopathic forms of TTP. The histologic features described above are not diagnostic of *idiopathic* TTP/HUS but can be seen in other forms of thrombotic microangiopathy, many of which are listed in Table 2.

Despite the relatively tight focus of this work (*idiopathic* TTP/HUS), there are a number of important limitations to this analysis because of the paucity of data in individual patients who meet the diagnostic criteria for *idiopathic* TTP/HUS, as discussed above.

Cross References

- ▶ Complement Regulation in the Kidney
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Scleroderma Renal Crisis

References

Baehr G, Klemperer P, Schifrin A. An acute febrile anemia and thrombocytopenic purpura with diffuse platelet thromboses of capillaries and arterioles. Trans Assoc Am Physicians. 1936;65:43–58.

Bianchi V, Robles R, et al. Von Willebrand factor-cleaving protease (ADAMTS13) in thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura. Blood. 2002;100(2):710–3.

Blum S, Gattringer R, et al. The case: hemolysis and acute renal failure. Babesiosis. Kidney Int. 2011;80(6):681–3.

Booth KK, Terrell DR, et al. Systemic infections mimicking thrombotic thrombocytopenic purpura. Am J Hematol. 2011;86(9):743–51.

Brilliant SE, Lester PA, et al. Hemolytic-uremic syndrome without evidence of microangiopathic hemolytic anemia on peripheral blood smear. South Med J. 1996;89(3):342–5.

Cohen JA, Brecher ME, et al. Cellular source of serum lactate dehydrogenase elevation in patients with thrombotic thrombocytopenic purpura. J Clin Apher. 1998;13(1):16–9.

Daram SR, Philipneri M, et al. Thrombotic thrombocytopenic purpura without schistocytes on the peripheral blood smear. South Med J. 2005;98(3):392–5.
Froehlich-Zahnd R, George JN, et al. Evidence for a role of anti-ADAMTS13 autoantibodies despite normal ADAMTS13 activity in recurrent thrombotic thrombocytopenic purpura. Haematologica. 2012;97(2): 297–303.
Furlan M, Robles R, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med. 1998;339(22):1578–84.
George JN. How I treat patients with thrombotic thrombocytopenic purpura: 2010. Blood. 2010;116(20): 4060–9.
George JN. Diagnosis of thrombotic thrombocytopenic purpura-hemolytic syndrome in adults. UpToDate; 2011.
Howard MA, Williams LA, et al. Complications of plasma exchange in patients treated for clinically suspected thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Transfusion. 2006; 46(1):154–6.
Laszik ZG, Silva F. Hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and other thrombotic microangiopathies. In: Jennette JC, Olson JL, Schwartz MM, Silva FG, editors. Heptinstall's pathology of the kidney. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 701–64.
Leung L. Clinical features, diagnosis, and treatment of disseminated intravascular coagulation in adults. UpToDate; 2010.
Moschcowitz E. An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries: an undescribed disease. 1925. Mt Sinai J Med. 2003;70(5):352–5.
Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(17):1676–87.
Raife T, Atkinson B, et al. Severe deficiency of VWF-cleaving protease (ADAMTS13) activity defines a distinct population of thrombotic microangiopathy patients. Transfusion. 2004;44(2):146–50.
Remuzzi G. HUS and TTP: variable expression of a single entity. Kidney Int. 1987;32(2):292–308.
Tsai HM. Pathophysiology of thrombotic thrombocytopenic purpura. Int J Hematol. 2010;91(1):1–19.
Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med. 1998;339(22): 1585–94.
Vesely SK, George JN, et al. ADAMTS13 activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: relation to presenting features and clinical outcomes in a prospective cohort of 142 patients. Blood. 2003;102(1):60–8.
Wolf G. Not known from ADAM(TS-13)–novel insights into the pathophysiology of thrombotic microangiopathies. Nephrol Dial Transplant. 2004; 19(7):1687–93.
Wu H, Birmingham DJ, et al. D-dimer level and the risk for thrombosis in systemic lupus erythematosus. Clin J Am Soc Nephrol. 2008;3(6):1628–36.
Zheng XL, Wu HM, et al. Multiple domains of ADAMTS13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura. Haematologica. 2010;95(9):1555–62.

Kupffer Cells in Immune Tolerance

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Synonyms

Kupffer cells: resident liver macrophages

Definition

Kupffer cells are an abundant population of macrophages found mostly in hepatic sinusoids.

Introduction

Kupffer cells are an abundant population of macrophages that reside in the sinusoids of the liver. They are intravascular and, therefore, perfectly located to phagocytose particulates from the blood. The location of the liver in the vascular system results in it receiving both antigenic material and innate immune stimulatory molecules from the intestine, via the hepatic portal vein. Both normal dietary constituents and highly reactive contaminants reach the liver, but in most cases an immune response would serve no useful purpose. For this reason, immune responses in the liver appear to be biased towards immune tolerance. Similarly, the presence of trace amounts of bacterial endotoxin in the portal blood results in a low level of continuous stimulation of innate immune pattern-recognition receptors, such as Toll-like receptor 4. This

influence affects the immunobiology of the liver, resulting in the local expression of adhesion molecules and the constitutive sequestration of activated T cells from the circulation.

The bias of the liver towards immune tolerance is most dramatically illustrated in the case of liver allografts. In mice, in some strain combination of rats and in pigs, an allogeneic liver transplant is frequently accepted. Even in humans, where liver transplantation is typically undertaken in the context of inflammatory disease, there is a bias towards allograft acceptance, and some patients can be weaned off immunosuppression. In addition, several important infections reside in the liver and are subject to ineffective immunity. One interpretation of this is that such pathogens, including hepatitis C virus (HCV), have evolved to take advantage of the liver's tolerogenic bias.

Many different populations of liver cells have been ascribed a role in liver tolerance. These include the hepatocytes, liver sinusoidal endothelial cells, hepatic stellate cells (also termed Ito cells), and the liver's resident and transient dendritic cells. This chapter considers specifically the role of Kupffer cells in inducing immune tolerance. As members of the monocyte-macrophage lineage, Kupffer cells are equipped both to drive inflammatory immunity and to suppress it. In many circumstances, Kupffer cell-mediated immunosuppression is an important element of liver tolerance.

The Origin of Kupffer Cells

Most populations of tissue macrophages are directly derived from circulating monocytes. A subset of macrophages, in particular the microglia of the central nervous system and the Langerhans cells of the skin, have a different origin. These cells arise from fetal precursors, are self-renewing in situ, and do not under normal conditions undergo repopulation from the blood (Hashimoto et al. 2011). Kupffer cells occupy intermediate ground. A subset of Kupffer cells is relatively resistant to ionizing radiation and host-type cells survive in a radiation bone marrow chimera (Kennedy and Abkowitz 1997). Conversely, around half of the liver cells that express mature macrophage markers are in fact bone marrow derived. This same proportion reflects the donor-derived Kupffer cells that persist in a transplanted liver. Therefore, it is likely that the normal liver contains two distinct populations of mature Kupffer cells, one a mainstream tissue macrophage and the other sessile and radioresistant (Klein et al. 2007). It is not yet clear whether these two subsets play different roles in liver immunity and immunopathology.

Immune Suppression Mediated by Cell-Cell Interactions

Kupffer cells express MHC class I and class II molecules, together with co-stimulatory molecules including CD40, CD80, and CD86. They are therefore able to present antigens to both CD4+ and CD8+ T cells. In short-term tissue culture, Kupffer cells activate CD8+ T cells causing their proliferation. They were also capable of cross-presentation of hepatocyte antigens in a coculture (Ebrahimkhani et al. 2011). The expression of MHC class II renders the cells capable of CD4+ T cell activation, but this has not been the topic of extensive study. Stimulation of Kupffer cells via TLR3 increases their expression of both MHC class II and co-stimulatory molecules, suggesting that innate immune activation may modulate their capacity to present antigen (You et al. 2008).

In addition to the capacity to present antigen, Kupffer cells also express a variety of inhibitory ligands that can inactivate or induce apoptosis in T cell that recognize them. One clear example is Fas ligand (CD95L), a tumor necrosis factor (TNF)-like cell surface molecule that engages its counter-receptor Fas (CD95) on activated T cells, leading to caspase-dependent T cell death. In other tissues, the expression of FasL has been linked to immune privilege in the anterior chamber of the eye and to the resistance of immunogenic melanomas to T cell killing (Elzey et al. 2001; Hahne et al. 1996). During

liver transplantation, the induction of tolerance is accompanied by an influx of T cells, followed by apoptotic death of those T cells (Qian et al. 1997). Kupffer cells recovered from such livers express elevated levels of FasL and can kill T cells that recognize them. Furthermore, the adoptive transfer of such Kupffer cells can prolong liver graft survival in a graft rejection model (Sun et al. 2003). Kupffer cells may be paralyzed using an injection of gadolinium chloride, and this treatment reduced the apoptosis of graft-infiltrating T cells and compromised graft survival. This effect was accompanied by loss of NF-kappaB activity and loss of FasL expression by the Kupffer cells and loss of their ability to induce T cell apoptosis (Chen et al. 2008). These and other lines of evidence suggest that FasL on Kupffer cells engages Fas on T cells, promoting T cell death and liver graft survival.

The co-stimulatory molecules CD80 (B7.1) and CD86 (B7.2) belong to a family that also contains an inhibitory molecule, programmed death-ligand 1 (PD-L1, also known as B7-H1). The protein engages a specific counter-receptor PD-1 that is expressed on activated T cells, and this interaction leads to T cell inactivation. Despite the molecule's name, the outcome is often not T cell death, but T cell exhaustion, a functionally compromised but potentially reversible state. Indeed, such PD-1-expressing exhausted T cells can be reinvigorated and can deliver antiviral immunity, simply by blocking the PD-L1/PD-1 interaction (Barber et al. 2006). PD-L1 is expressed on Kupffer cells and appears to mediate tolerance. Thus, Kupffer cells associated with hepatocellular cancers express a high level of PD-L1, and this is correlated with a poor outcome. Such PD-L1 high Kupffer cells were associated with PD-1-high, functionally incapable CD8+ T cells, while the PD-L1 expression was induced by IL-10, secreted by the cancer cells (Wu et al. 2009). The coordinated upregulation of IL-10 and PD-L1 may also be an immunosuppressive strategy adopted by HCV. Thus, the core protein of HCV binds specifically to Toll-like receptor-2 (TLR-2), inducing the secretion of the inflammatory cytokines interleukin-1 beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) but inhibiting others (interferon-alpha and interferon-beta). In parallel, HCV core induces both IL-10 and PD-L1 (Tu et al. 2010). This interaction could therefore compromise antiviral immunity in two ways: suppression of a key antiviral cytokine, IFN-alpha, and the induction of T cell exhaustion by PD-L1. Both mechanisms are supported by clinical observation. Exhausted PD-1 expressing T cells are in fact found in HCV (Radziewicz et al. 2007), while exogenous IFN-alpha is the mainstay of anti-HCV therapy.

Immune Suppression Mediated by Cytokines

Secretion of IL-10 underpins many examples of liver tolerance. Human Kupffer cells exposed to endotoxin secrete IL-10, and this regulates the secretion of inflammatory IL-6 and TNF-alpha by the same cells (Knolle et al. 1995). Kupffer cell-derived IL-10 also acts in trans, negatively regulating the IL-12- and IL-18-dependent activation of liver NK cells (Tu et al. 2008). Kupffer cells may also regulate the severity of virus infections via IL-10. The infection of human Kupffer cells with either wild-type or attenuated yellow fever virus resulted in a cytokine cascade, but in the case of the attenuated virus, more IL-10 was secreted and less of inflammatory cytokines such as TNF-alpha (Woodson et al. 2011). Therefore, a lack of IL-10 may explain the immunopathogenesis associated with wild-type yellow fever virus infection. Similarly, in a mouse model of acute liver injury induced by the injection of the plant lectin, Concanavalin-A, secretion of IL-10 by Kupffer cells, was important in limiting injury (Erhardt et al. 2007). Kupffer cell IL-10 therefore broadly contains liver injury.

In addition to its antagonism of IL-18 and promotion of PD-L1 expression, IL-10 also engages in cross-talk with other immunosuppressive cytokines. Transforming growth factor-beta1 (TGF-beta1) is expressed on apoptotic cell fragments, and such fragments interact with Kupffer cells to induce IL-10 and suppress the

production of inflammatory cytokines including TNF-alpha (Zhang et al. 2011). Just as HCV induces IL-10, so hepatitis B virus (HBV) infects Kupffer cells resulting in the secretion of TGF-beta1 (Li et al. 2012). Thus, Kupffer cells synthesis and response to TGF-beta1, their secretion of IL-10 and their expression of PD-L1 form an interconnected network of immunosuppressive signals, which may both protect the liver from immunopathology and create a window of vulnerability to viral pathogens. Kupffer cells do not simply make IL-10 themselves; they also induce its synthesis by regulatory T cells (Breous et al. 2009), thus participating in active antigen-specific immunosuppression.

Immune Suppression Mediated by Other Mechanisms

In addition to cytokines, Kupffer cells secrete diverse other immunosuppressive molecules. IFN-gamma induces nitric oxide (NO) synthase in Kupffer cells, and this molecule can suppress T cell clonal proliferation (Roland et al. 1994). Kupffer cell activation also results in the upregulation of the enzymes phospholipase A2 and cyclooxygenase A2, which result in synthesis and secretion of the immunosuppressive prostaglandin, PGE2 (Perez et al. 1997).

The enzymatic properties of Kupffer cells further contribute to their immunosuppressive function. The enzyme indoleamine 2,3 dioxygenase (IDO) is implicated in placental tolerance of the fetal allograft, where it acts by creating a tryptophan-deficient local milieu that inhibits T cells activation (Munn et al. 1998). In Kupffer cells, IFN-gamma treatment increases IDO expression and tryptophan depletion, with resulting immunosuppression that could be reversed by the inhibition of IDO (Yan et al. 2010). In another level of regulation, the expression of the enzyme arginase in the liver results in a low L-arginine environment. In an arginine-depleted environment, Kupffer cells secrete increased PGE2, and this causes immunosuppression (Callery et al. 1991).

Conclusion

Kupffer cells exemplify the aspect of macrophage function that deals with homeostasis. Their responses to the inflammatory cytokine IFN-gamma are to secrete TNF-alpha and IL-6, but also to secrete IL-10, and to express NO synthase and IDO, all of which promote immunosuppression. In addition, activated Kupffer cells suppress NK cells, but enhance IL-10 production by regulatory T cells. Similarly, Kupffer cells respond to activation signals by secreting PGE2, which suppresses T cell activation. With all these anti-inflammatory mechanisms, Kupffer cells appear strongly biased towards the containment of injury and the moderation of local immunity. Even the classical "Kupffer cell hyperplasia" described in HCV-infected liver may reflect the containment, rather than the promotion of liver cell injury (Sitia et al. 2011). This bias is likely to be an adaptation to the exposure of Kupffer cells to diverse nonself and pathogen-associated molecules that originate in the intestinal microbiota, but diverse pathogens have evolved mechanisms to exploit Kupffer cells. One of the issues that remains to be addressed is whether Kupffer cell-mediated liver tolerance is sometimes too much of a good thing. The accessibility of Kupffer cells within the circulation renders them potential targets for therapy (Yang et al. 2008), so it will be possible to both enhance and inhibit Kupffer cell-driven immune tolerance for therapeutic benefit.

Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Adaptive Immune Cells in the Liver
- ▶ Animal Models of Hepatitis B and C
- ▶ B7 and CD28 Families
- ▶ Cell Adhesion Molecules
- ▶ Fas/Fas Ligand
- ▶ Hepatic Lymphatic System
- ▶ Immune Responses to the Hepatitis C Virus
- ▶ Innate Immune Cells in the Liver

- ▶ Liver Transplantation for Chronic Viral Hepatitis
- ▶ Liver Transplantation Tolerance in Animal Models for Encyclopedia of Medical Immunology
- ▶ Liver Vasculature and Microvasculature
- ▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance
- ▶ Nitric Oxide
- ▶ Primary T-Cell Activation in Liver
- ▶ Tregs in the Liver
- ▶ Tumor Macrophages
- ▶ Ultrastructure of the Liver Sinusoid

References

Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439:682–7.

Breous E, Somanathan S, Vandenberghe LH, Wilson JM. Hepatic regulatory T cells and Kupffer cells are crucial mediators of systemic T cell tolerance to antigens targeting murine liver. Hepatology. 2009;50:612–21.

Callery MP, Mangino MJ, Flye MW. Arginine-specific suppression of mixed lymphocyte culture reactivity by Kupffer cells–a basis of portal venous tolerance. Transplantation. 1991;51:1076–80.

Chen Y, Liu Z, Liang S, Luan X, Long F, Chen J, Peng Y, Yan L, Gong J. Role of Kupffer cells in the induction of tolerance of orthotopic liver transplantation in rats. Liver Transpl: Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc. 2008;14:823–36.

Ebrahimkhani MR, Mohar I, Crispe IN. Cross-presentation of antigen by diverse subsets of liver cells. Hepatology. 2011;54:1379.

Elzey BD, Griffith TS, Herndon JM, Barreiro R, Tschopp J, Ferguson TA. Regulation of fas ligand-induced apoptosis by tnf. J Immunol. 2001;167:3049–56.

Erhardt A, Biburger M, Papadopoulos T, Tiegs G. IL-10, regulatory T cells, and Kupffer cells mediate tolerance in concanavalin A-induced liver injury in mice. Hepatology. 2007;45:475–85.

Hahne M, Rimoldi D, Schroter M, Romero P, Schreier M, French LE, Bornand T, Fontana A, Lienard D, Cerottini J. Melanoma cell expression of Fas(Apo-1/CD95) ligand: implications for tumor immune escape [see comments]. Science. 1996;274:1363–6.

Hashimoto D, Miller J, Merad M. Dendritic cell and macrophage heterogeneity in vivo. Immunity. 2011;35:323–35.

Kennedy DW, Abkowitz JL. Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. Blood. 1997;90:986–93.

Klein I, Cornejo JC, Polakos NK, John B, Wuensch SA, Topham DJ, Pierce RH, Crispe IN. Kupffer cell heterogeneity: functional properties of bone marrow derived and sessile hepatic macrophages. Blood. 2007;110:4077–85.

Knolle P, Schlaak J, Uhrig A, Kempf P, Meyer zum Buschenfelde KH, Gerken G. Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J Hepatol. 1995;22:226–9.

Li H, Zheng HW, Chen H, Xing ZZ, You H, Cong M, Jia JD. Hepatitis B virus particles preferably induce Kupffer cells to produce TGF-beta1 over pro-inflammatory cytokines. Dig Liver Dis: Off J Ital Soc Gastroenterol Ital Assoc Study Liver. 2012;44:328–33.

Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science. 1998;281:1191–3.

Perez RV, Swanson C, Morgan M, Erickson K, Hubbard NE, German JB. Portal venous transfusion up-regulates Kupffer cell cyclooxygenase activity: a mechanism of immunosuppression in organ transplantation. Transplantation. 1997;64:135–9.

Qian S, Lu L, Fu F, Li Y, Li W, Starzl TE, Fung JJ, Thomson AW. Apoptosis within spontaneously accepted mouse liver allografts: evidence for deletion of cytotoxic T cells and implications for tolerance induction. J Immunol. 1997;158:4654–61.

Radziewicz H, Ibegbu CC, Fernandez ML, Workowski KA, Obideen K, Wehbi M, Hanson HL, Steinberg JP, Masopust D, Wherry EJ, et al. Liver-infiltrating lymphocytes in chronic human hepatitis C virus infection display an exhausted phenotype with high levels of PD-1 and low levels of CD127 expression. J Virol. 2007;81:2545–53.

Roland CR, Walp L, Stack RM, Flye MW. Outcome of Kupffer cell antigen presentation to a cloned murine Th1 lymphocyte depends on the inducibility of nitric oxide synthase by IFN-gamma. J Immunol. 1994;153:5453–64.

Sitia G, Iannacone M, Aiolfi R, Isogawa M, van Rooijen N, Scozzesi C, Bianchi ME, von Andrian UH, Chisari FV, Guidotti LG. Kupffer cells hasten resolution of liver immunopathology in mouse models of viral hepatitis. PLoS Pathog. 2011;7:e1002061.

Sun Z, Wada T, Maemura K, Uchikura K, Hoshino S, Diehl AM, Klein AS. Hepatic allograft-derived Kupffer cells regulate T cell response in rats. Liver Transpl: Off Publ Am Assoc Study Liver Dis Int Liver Transpl Soc. 2003;9:489–97.

Tu Z, Bozorgzadeh A, Pierce RH, Kurtis J, Crispe IN, Orloff MS. TLR-dependent cross talk between human Kupffer cells and NK cells. J Exp Med. 2008;205:233–44.

Tu Z, Pierce RH, Kurtis J, Kuroki Y, Crispe IN, Orloff MS. Hepatitis C virus core protein subverts the

antiviral activities of human Kupffer cells. Gastroenterology. 2010;138:305–14.

Woodson SE, Freiberg AN, Holbrook MR. Differential cytokine responses from primary human Kupffer cells following infection with wild-type or vaccine strain yellow fever virus. Virology. 2011;412:188–95.

Wu K, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. Cancer Res. 2009;69:8067–75.

Yan ML, Wang YD, Tian YF, Lai ZD, Yan LN. Inhibition of allogeneic T-cell response by Kupffer cells expressing indoleamine 2,3-dioxygenase. World J Gastroenterol: WJG. 2010;16:636–40.

Yang SC, Bhide M, Crispe IN, Pierce RH, Murthy N. Polyketal copolymers: a new acid-sensitive delivery vehicle for treating acute inflammatory diseases. Bioconjug Chem. 2008;19:1164–9.

You Q, Cheng L, Kedl RM, Ju C. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. Hepatology. 2008;48:978–90.

Zhang M, Xu S, Han Y, Cao X. Apoptotic cells attenuate fulminant hepatitis by priming Kupffer cells to produce interleukin-10 through membrane-bound TGF-beta. Hepatology. 2011;53:306–16.

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Lichen Planopilaris

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Synonyms

Cicatricial alopecia; Follicular lichen planus

Definition

Cicatricial or scarring alopecia includes rare disorders that permanently destroy the hair follicle and replace it by scar tissue and cause permanent hair loss. Lichen planopilaris is a primary lymphocytic scarring alopecia, whereas in secondary scarring alopecia, alopecia is a feature of systemic disease as in discoid lupus erythematosus, scleroderma, and dermatomyositis.

Introduction

Lichen planopilaris (LPP) is a variant of lichen planus selectively involving hair follicles with a lymphocytic inflammatory process that eventually destroys the pilosebaceous unit permanently resulting in scarring alopecia. LPP is the most common cause of adult primary scarring alopecia. Frontal fibrosing alopecia and Lasseur-Graham-Little-Piccardi syndrome are considered variants of LPP.

Pathophysiology

Pathogenesis of LPP is poorly understood. The reaction is mediated by T lymphocytes (CD4 and CD8) activated by Langerhans cells that are increased in dermis and epidermis. The target follicular antigens are unknown. In lichen planus, T lymphocytes destroy keratinocytes, which express an unknown antigen on their surface. In LPP, T lymphocytes are located around the upper part of the hair follicle around the infundibulum and isthmus destroying the bulge area, which is a specialized portion of hair follicle marked by the insertion of arrector pili muscle. The bulge area cells are stem cells which generate the new lower anagen hair follicle. Destruction of the hair follicle stem cells in the hair bulge and the sebaceous glands leads to permanent hair loss. In contrast, in alopecia areata, the inflammatory infiltrate is located around the hair bulb and the hair follicle stem cells and sebaceous glands are not affected; hence, the hair loss is always potentially reversible.

The cell-mediated reaction may be triggered by an endogenous or exogenous agent, such as virus, medications, or contact sensitizers, which bind to keratinocytes and follicular epithelium,

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resulting in production of cytokines and chemotactic factors causing inflammation. Contact sensitizers like gold or mercury can act as haptens and evoke inflammation. Certain viruses like hepatitis C virus and human immunodeficiency virus and drugs like beta-blockers, quinidine, and thiazides can cause lichenoid eruptions, which exhibit similar cutaneous and histological findings as lichen planus (Kang et al. 2008). Lichen planus and LPP show similarities in pathogenesis; however, the target antigen in both diseases remains unknown.

The recent work suggests LPP is a lipid metabolic disorder caused by a downregulation of the peroxisome proliferator-activated receptor gamma (PPAR-gamma).

Gene expression profiling studies have shown that the expression of genes required for lipid metabolism and peroxisome biogenesis is decreased in LPP. The expression of PPAR-gamma, a transcription factor that regulates both inflammatory and lipid metabolic genes, has been found dramatically downregulated in LPP (Karnik et al. 2009). This loss of PPAR-gamma function leads to decreased peroxisome biogenesis and lipid homeostasis, causing tissue damage (lipotoxic effects) of the pilosebaceous unit. This tissue damage then triggers chemokine and cytokine expression and activates a lipid-mediated programmed cell death (lipoapoptosis). The combination of genetic and environmental triggers may be leading to this localized and acquired PPAR-gamma dysfunction. Based on this hypothesis, PPAR-gamma agonist therapy has been used in the treatment of LPP (Mirmirani and Karnik 2009).

Clinical Features

LPP is more common in women than in men. The age of onset is frequently between 30 and 60 years. It is unusual in children with incidence of 0.021 % of the total number of new pediatric dermatology outpatient in a hospital in India (Handa and Sahoo 2002). Its incidence rate in the United States ranges from 1.15 % to 7.59 % among all new patients with hair loss (Chiang et al. 2010). The University of British Columbia, Canada, found a lower incidence in the Asian population compared to other ethnic groups (Tan et al. 2004).

The course of the disease is variable. It may evolve slowly with one or more patches of hair loss or diffuse central thinning, with slow progression over the years. In other cases, the course is rapid, and within a few months, patchy hair loss or diffuse thinning spreads over large areas of the scalp. Symptoms of itching, burning, pain, and tenderness are often severe. Few patients are asymptomatic. The scalp lesions may be single or multiple and focal or diffuse and can occur anywhere on the scalp more commonly over the vertex. The center is smooth, devoid of follicular markings, and the margins show perifollicular erythema and perifollicular scale. This is in contrast to DLE where the follicular plugging is in the center of the active patches. A hair pull test may yield anagen hair, when present this is a useful sign of disease activity. Cutaneous, nail, and mucous membrane LP may occur before, during, or after the onset of scalp lesions. The occurrence of associated LP varies in reports from 17 % to 50 % of patients with LPP (Price 2011).

Diagnosis

Scalp biopsy helps to differentiate LPP from other cicatricial alopecias especially discoid lupus erythematosus (DLE). During active disease, biopsy should be taken from the active margin showing perifollicular erythema and scaling.

Histopathology

In early stages, a lichenoid lymphocytic infiltrate affecting the infundibulum and isthmus is observed and sparing the lower portion of hair follicle. Thereafter, interface dermatitis is seen with loss of the basement membrane zone. Sebaceous glands are lost in the early lesions and the root sheath of follicles is destroyed. Mucinous perifollicular fibroplasia with absence of the interfollicular dermal mucin in the upper dermis has been described

in vertical sections. Finally, follicular structure is replaced with extensive perifollicular lamellar fibrosis, especially around the isthmus, and the lichenoid infiltrate "backs away" from the follicle. Direct immunofluorescence may show positive colloid body staining with anti-immunoglobulin M (IgM), anti-IgA, anti-IgG, or C3 at the dermoepidermal junction or around the infundibulum. There is a "shaggy" or linear band of fibrinogen deposition along the basement membrane zone of affected follicles, while the interfollicular epidermis is negative for immunoreactants (Kang et al. 2008; Stefanato 2010).

Treatment

LPP is a very challenging disease to treat. The role of treatment is currently limited to relieve symptoms and to control or slow further progression of disease. Currently available treatments are sometimes of limited efficacy, and there is a demand for novel therapies. Various forms of treatment regimens have been developed based on the severity of disease, age of the patient, and the physician experience. Hair loss is irreversible, and consequently, rapidly progressive disease is treated aggressively.

Corticosteroids

In mild to moderate disease, potent topical corticosteroids with or without intralesional triamcinolone acetonide 10 mg/ml for a total of 2 ml every 4–6 weeks are recommended. Topical steroids like fluocinolone acetonide in an oil formulation (Derma-Smoothe) or clobetasol lotion reduce inflammation and relieve symptoms in most patients. Oral corticosteroids are reserved for rapidly progressing disease. Prednisone 1 mg/kg/day tapered over 2–3 months can be prescribed in severe disease and can be used as a bridge therapy until other agents show effectiveness.

Tetracyclines

Tetracycline antibiotics are commonly used in LPP because of their safety profile; however, there is weak evidence to support its effectiveness (Spencer et al. 2009). Tetracycline is used in a dosage of 1 g/day or doxycycline 100 mg twice a day for a period of 3–6 months (Spencer et al. 2009).

Hydroxychloroquine

Hydroxychloroquine is an antilymphocytic, antimalarial drug that has been widely used in dermatology since the 1950s. It is generally well tolerated. The exact mode of action of hydroxychloroquine is unclear. It possibly interferes with antigen presentation and production of cytokines such as tumor necrosis factor-alpha and interferon-gamma. The efficacy of hydroxychloroquine in decreasing symptoms and signs in LPP was recently reported in an evidence-based study (Chiang et al. 2010). It is significantly effective after 6–12 months of treatment, in decreasing symptoms and signs of LPP.

Mycophenolate Mofetil

Mycophenolate mofetil has been successfully used for the treatment of LLP which is resistant to treatment with hydroxychloroquine. It specifically inhibits activated lymphocytes which play a key role in the inflammatory process underlying LPP. The recommended dosage for LPP is 500 mg twice daily for 4 weeks and then 1 g twice daily for 6 months, some patients may need to continue for up to 1 year (Cho et al. 2010).

Cyclosporin

Cyclosporin has been used in severe, refractory cases of LPP. Its use is limited by its side effects. Cyclosporine is a calcineurin inhibitor that acts by suppressing gene transcription of IL-2, which is required for activation and proliferation of T cells responsible for the T cell-mediated immune response in lichen planus. It is used in a dose of 3–5 mg/kg. It is helpful in rapidly progressive LPP but is associated with a high relapse rate (Mirmirani et al. 2003; Sperling and Nguyen 2010).

Miscellaneous

Various other systemic agents used in the treatment of lichen planus have been used in LPP

with marginal efficacy. Systemic retinoids, griseofulvin, thalidomide, dapsone, topical tacrolimus, and minoxidil have been used with little evidence-based support (Sperling and Nguyen 2010).

Newer Therapies

PPAR-Gamma Agonist

Based on new insight into pathogenesis of LPP, PPAR-gamma agonists have been used in the treatment of LPP. They exert their action by increasing the activity of the nuclear receptor PPAR-gamma. Thiazolidinediones or glitazones are medications used for treatment of type 2 diabetes mellitus. They have been shown to have anti-inflammatory, antiproliferative, and immunomodulatory effects including downregulation of proinflammatory nuclear transcription factors and inflammatory interleukins and other inflammatory molecules. When compared with other oral medications used for treatment of LP, thiazolidinediones have an acceptable adverse effects and safety profile. Pioglitazone hydrochloride 15 mg/day has been used for 8 months with good clinical response (Mirmirani and Karnik 2009). Some side effects limiting use of this drug include body weight gain, congestive heart failure, bone fractures, and possibly bladder cancer which is most likely related to cumulative dose (Cariou et al. 2012; Neumann et al. 2012). The dosage of pioglitazone used in treatment of LPP is relatively smaller and time duration much shorter compared to its use in diabetes mellitus. Another consideration for drug delivery is the topical use due to its concern of adverse effects.

Biological Agents

A recent report has shown efficacy of rituximab in a patient with juvenile arthritis and refractory LPP; however, as with other systemic medications, risk-benefit ratio has to be considered before using newer medications. Rituximab is a chimeric monoclonal antibody directed against CD20 and has been suggested for patients who have autoimmune diseases (Erras et al. 2011).

Laser

Low-dose excimer 308-nm laser has been shown effective in LPP. Twice weekly treatment helped in relieving symptoms and decreased erythema and hyperkeratosis. The probable mode of action is T cell depletion and alteration in cytokine expression (Navarini et al. 2011).

LPP still remains a therapeutic challenge. Early diagnosis and treatment is the key in limiting disease progression. The choice of treatment is based on the extent of disease, symptoms, and progression of hair loss. In general, first-line treatment for active disease includes use of topical and intralesional corticosteroids, topical tacrolimus, oral antibiotics, and hydroxychloroquine sulfate. For more symptomatic, active, and rapidly advancing disease, or disease that is recalcitrant to treatment, medications such as oral prednisone, mycophenolate mofetil, and cyclosporine are used. Lichen Planopilaris Activity Index (LPPAI) score was recently introduced as a numeric summary of symptoms and signs. It allows statistical comparison of disease activity pre- and posttreatment and is helpful in patient follow-up (Chiang et al. 2010).

Cross-References

- ▶ Alopecia Areata
- ▶ Environment and Autoimmunity
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Cariou B, Charbonnel B, Staels B. Thiazolidinediones and PPAR gamma agonists: time for a reassessment. Trends Endocrinol Metab. 2012;23(5):205–15.

Chiang C, et al. Hydroxychloroquine and lichen planopilaris: efficacy and introduction of lichen planopilaris activity index scoring system. J Am Acad Dermatol. 2010;62(3):387–92.

Cho BK, et al. Efficacy and safety of mycophenolate mofetil for lichen planopilaris. J Am Acad Dermatol. 2010;62(3):393–7.

Erras S, et al. Rapid and complete resolution of lichen planopilaris in juvenile chronic arthritis treated with rituximab. Eur J Dermatol. 2011;21(1):116–7.

Handa S, Sahoo B. Childhood lichen planus: a study of 87 cases. Int J Dermatol. 2002;41(7):423–7.
Kang H, et al. Lichen planopilaris. Dermatol Ther. 2008;21(4):249–56.
Karnik P, et al. Hair follicle stem cell-specific PPARgamma deletion causes scarring alopecia. J Invest Dermatol. 2009;129(5):1243–57.
Mirmirani P, Karnik P. Lichen planopilaris treated with a peroxisome proliferator-activated receptor gamma agonist. Arch Dermatol. 2009;145(12):1363–6.
Mirmirani P, Willey A, Price VH. Short course of oral cyclosporine in lichen planopilaris. J Am Acad Dermatol. 2003;49(4):667–71.
Navarini AA, et al. Low-dose excimer 308-nm laser for treatment of lichen planopilaris. Arch Dermatol. 2011;147(11):1325–6.
Neumann A, et al. Pioglitazone and risk of bladder cancer among diabetic patients in France: a population-based cohort study. Diabetologia. 2012;55:1953–62.
Price, VMP. Cicatricial alopecia. In: Price V, editor. An approach to diagnosis and management. New York: Springer; 2011;17–19.
Spencer LA, Hawryluk EB, English 3rd JC. Lichen planopilaris: retrospective study and stepwise therapeutic approach. Arch Dermatol. 2009;145(3):333–4.
Sperling LC, Nguyen JV. Commentary: treatment of lichen planopilaris: some progress, but a long way to go. J Am Acad Dermatol. 2010;62(3):398–401.
Stefanato CM. Histopathology of alopecia: a clinicopathological approach to diagnosis. Histopathology. 2010;56(1):24–38.
Tan E, et al. Primary cicatricial alopecias: clinicopathology of 112 cases. J Am Acad Dermatol. 2004;50(1):25–32.

Lichen Planus

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Synonyms

Lichen planopilaris (LPP); Lichen planus (LP); Oral lichen planus (OLP)

Definition

Lichen planus is an inflammatory disease which can involve hair, skin, nails, and mucosa. Its occurrence may be completely idiopathic and related to medications or hepatitis C (Boyd and Neldner 1991).

Introduction

Lichen planus (LP) is inflammatory in nature and was first described by Hebra as "leichen ruber." Later, in 1869, Erasmus Wilson named the disease lichen planus. Skin, hair, nail, and mucosal surfaces may be affected by LP (Boyd and Neldner 1991).

Epidemiology

Cutaneous LP is believed to affect less than one percent of the population (Boyd and Neldner 1991). Between 1 % and 4 % of the population has been found to have evidence of oral lichen planus (OLP). LP usually presents in the fifth and sixth decades. OLP is diagnosed at a mean age of 52. In some studies, women have been found to be affected by LP more often than men. Up to 75 % of cutaneous LP patients have been found to have oral lesions, while between 10 % and 20 % of OLP patients develop cutaneous LP (Shiohara and Kano 2008).

Pathophysiology/Immunology

All forms of lichen planus are believed to be part of an immune-mediated process. With regard to immunopathogenesis, most data exists on OLP. Multiple mechanisms have been implicated in the immune dysregulation of OLP. These include humoral immunity, autoimmune response, cell-mediated immune response, and nonspecific mechanisms (Roopashree et al. 2010).

The cell-mediated immune response associated with OLP involves auto-cytotoxic CD 8 T cells which set off basal cell apoptosis in the oral epithelium (Lavanya et al. 2011). The initiating event is believed to be keratinocyte antigen expression or antigen unmasking, which may be triggered by medications, allergens, trauma, infection, or another unknown cause (Ismail et al. 2007).

The exact antigen is unknown but is thought to possibly be a self-peptide. Heat shock proteins, which are upregulated in OLP, are thought to possibly be candidates for the role of the antigen in OLP (Roopashree et al. 2010). Subsequently, T cells, primarily CD 8+ cells, enter the epithelium and become activated. Activation may occur through activated CD 4+ lymphocytes or binding of antigen to major histocompatibility complex (MHC-1) located on the keratinocyte (Lavanya et al. 2011). It is believed that Langerhans cells may initiate the local immune response in OLP (Ismail et al. 2007). In lesions of OLP, there are increased Langerhans cells and upregulation of the expression of MHC II. CD 4+ T helper cells are activated through antigen presentation and IL-12. These CD 4+ T helper cells activate CD 8+ T cells with interferon gamma (INF gamma), IL-12, and receptor interaction (Lavanya et al. 2011). Subsequently, basal keratinocytes are killed through tumor necrosis factor (TNF)-alpha-induced, FAS-FAS-L-mediated, or granzyme-B-activated apoptosis (Lavanya et al. 2011).

It has also been proposed that autoimmunity may be responsible for OLP. TGF-B1, which is immunosuppressive, is deficient in OLP. In addition, there is an immune privilege breakdown in OLP in the form of a lack of T cell apoptosis induced by keratinocytes. In OLP, antigen-presenting cells (APCs) are involved in the endocytosis of apoptotic basal keratinocytes. Subsequently, these APCs mature and may activate an autoreactive T cell response against basal keratinocytes. Finally, heat shock proteins, which may serve as self-antigens in OLP, also further the hypothesis that an autoimmune reaction is involved (Roopashree et al. 2010).

The nonspecific immune mechanisms involved in OLP are thought to promote the migration of lymphocytes into the epithelium, enabling the damage of keratinocytes. Factors involved in these nonspecific mechanisms include mast cells, chemokines, matrix metalloproteinases, and the basement membrane of the epithelium (Roopashree et al. 2010).

Although further studies are needed to characterize a humoral response in OLP, in one study circulating autoantibodies against desmoglein 1 and 3 were demonstrated (Roopashree et al. 2010; Lukac et al. 2006).

Presentation

Cutaneous

Classically, the lesions of cutaneous LP have been described as being pruritic, purple, polygonal, and planar papules. A fine white scale, referred to as Wickham's striae, may be present on top of these lesions. Wickham's striae represent an increased granular layer in the epidermis. In cutaneous disease, extremities, particularly wrists and lower legs, are most commonly involved. The neck, trunk, and back may also be involved (Boyd and Neldner 1991). Cutaneous LP is often intensely pruritic. The lesions of LP tend to koebnerize or spread to areas of physical trauma (Shiohara and Kano 2008). There are several variants of cutaneous disease which include hypertrophic, atrophic, linear, vesiculobullous, annular, palmoplantar, actinic, follicular, and lichen planus pemphigoides (Boyd and Neldner 1991).

Cutaneous LP tends to resolve spontaneously within a year (Le Cleach and Chosidow 2012). When lesions of lichen planus clear, they may leave postinflammatory hyperpigmentation, particularly on patients with darker skin types (Usatine and Tinitigan 2011).

Nails

Nail lichen planus often occurs in adults and may be seen with or without other cutaneous or mucosal disease. There is usually nail matrix involvement and nail thinning. Longitudinal ridging, fissuring, and pterygium may be seen. Other symptoms include onycholysis and subungual hyperkeratosis. More rarely, trachyonychia, nail atrophy, nail bed erosions, yellow nail syndrome-like changes, and pigment changes may be seen (Piraccini et al. 2010).

Oral

Lesions in oral lichen planus can vary. Reticular and erosive are the two main types of oral lichen planus (OLP). Reticular OLP is the most common

form and appears as an interwoven network of white lines with surrounding erythema. It appears on the buccal mucosa, the gingiva, mucobuccal fold, lips, palate, and tongue. Reticular OLP is generally asymptomatic. There is a plaque-like variant of reticular OLP often seen on the dorsal tongue. Erosive OLP presents with red and ulcerated lesions with peripheral keratotic striae. Erosive LP may appear in atrophic and bullous forms. Erosive OLP can cause pain and discomfort (Edwards and Kelsch 2002).

Those with OLP have been found to have approximately ten times the risk of developing squamous cell carcinoma compared to the general population. The erosive and atrophic forms are most commonly associated with a higher risk (Edwards and Kelsch 2002).

Other Mucosa

Lichen planus can also affect other mucosal surfaces including conjunctiva, esophagus, and genitalia. Patients with involvement of the penis, vulva, or vagina may present with pruritus, pain, burning, painful intercourse, or sexual dysfunction. Genital LP may result in vulvar scarring, vaginal stenosis, agglutination of labia minora, and phimosis (Le Cleach and Chosidow 2012).

Patients with esophageal involvement may complain of painful or difficulty swallowing. This may result in stricture, redness, and sloughing of the mucosa (Le Cleach and Chosidow 2012).

Lichen Planopilaris

Lichen planopilaris (LPP) is a follicular variant of LP. There is lymphocytic damage of the hair follicle which leads to a cicatricial alopecia. There are three subtypes. Classic disease involves the scalp and may also involve other cutaneous sites. Frontal fibrosing alopecia produces a band-like scarring alopecia along the anterior hairline and is most frequently seen in adult females. Graham-Little-Piccardi-Lassueur syndrome entails scarring hair loss in the scalp, non-scarring alopecia in the pubic area, and axillae and papular follicular involvement of LP in the skin (Kang et al. 2008).

Hepatitis C

There is a statistically significant association between hepatitis C virus (HCV) infection and the development of LP. The geographical location affects the association between HCV and LP (Shengyuan et al. 2009). The strongest association between HCV and lichen planus has been seen in Japanese and Mediterranean populations (Shiohara and Kano 2008). Patients with LP may be screened for HCV using enzyme-linked immunosorbent assay (ELISA) (Le Cleach and Chosidow 2012).

Medications

Drug-induced lichen planus may present similarly to other forms of lichen planus. Implicated medications include beta-blockers, penicillamine, quinidine, quinine, and methyldopa (Thompson and Skaehill 1994). Other medications that may be associated are nonsteroidal anti-inflammatory drugs (NSAIDS), penicillamine, angiotensin-converting enzyme inhibitors, beta-blockers, gold, lithium, and sulfonylurea drugs (Ellgehausen et al. 1998). Other broad categories of causative or exacerbating agents in OLP include antimalarials and antiretrovirals (Ismail et al. 2007). Dental products may also cause or exacerbate OLP. These include metals, composite and resin-based materials, and dental amalgam (Ismail et al. 2007).

Diagnosis

The diagnosis of lichen planus may be made on the basis of patient history and clinical presentation. Skin, hair, or mucosal biopsies of lesions may be done for confirmation.

In cutaneous LP, histology reveals a lichenoid band of lymphocytes located in the papillary dermis in addition to compact hyperkeratosis, hypergranulosis, irregular acanthosis with rete ridges that are sawtoothed, and degeneration of the basal layer. There is also melanin incontinence. On direct immunofluorescence, colloid

bodies stain for immunoglobulin, fibrin, and complement. The immunoglobulin consists mostly of IgM. The histology in OLP reveals more parakeratosis. The histology of LPP consists of plugged follicles surrounded by lichenoid lymphocytes (Rapini 2005).

Differential Diagnosis

The differential diagnosis for cutaneous LP includes other papulosquamous eruptions including secondary syphilis and psoriasis. Other disorders on the differential include lichen nitidus and lupus erythematosus. The differential for OLP is broad and includes leukoplakia, candidiasis, discoid lupus erythematosus, and secondary syphilis. Vaginal LP may mimic the appearance of bullous dermatoses, lichen sclerosus et atrophicus, or atrophic vaginitis (Boyd and Neldner 1991). Histologic studies often help to distinguish these various disorders.

Treatment and Management

Cutaneous Lichen Planus

Spontaneous resolution of cutaneous LP may occur between 1 and 2 years after the onset of disease (Usatine and Tinitigan 2011). Topical corticosteroids are considered to be first-line treatment for cutaneous disease. Specifically, high-potency topical steroids such as clobetasol or halobetasol are applied to individual lesions twice daily. Lesions may also be injected with intralesional kenalog (Usatine and Tinitigan 2011). Oral steroids are another alternative for treatment, particularly when lesions are unresponsive to topical or intralesional therapy (Le Cleach and Chosidow 2012). Oral retinoids are also an option. Oral acitretin may be used. Phototherapy also remains an option for therapy of cutaneous lichen planus. Newer treatments for LP include enoxaparin sodium (low-molecular-weight heparin), sulfasalazine, tetracycline, biologics, metronidazole, and thalidomide (Asch and Goldenberg 2011).

Oral Lichen Planus

Erosive OLP tends to be symptomatic necessitating treatment. As in cutaneous LP, topical corticosteroids are considered first-line therapy in the treatment of OLP. Unresponsive LP may require oral steroids. In addition, topical retinoids have shown efficacy in certain types of OLP (Le Cleach and Chosidow 2012).

Other Mucosa

In anogenital LP, prevention of scarring is the main therapeutic goal. High-potency topical corticosteroids may be helpful. In addition, vaginal dilators and penile foreskin retraction may help to prevent synechiae. Adhesions may require surgical intervention (Le Cleach and Chosidow 2012).

Nail Lichen Planus

Intralesional or systemic steroids are usually the treatment of choice in nail LP (Piraccini et al. 2010).

Lichen Planopilaris

Treatment of LPP is difficult. Generally, treatment serves to decrease pain and pruritus, halt disease progression, and stop development of further hair loss. Generally, hair regrowth of this cicatricial alopecia is not possible, except in early disease (Assouly and Reygagne 2009).

First-line treatment includes topical therapy with ultra-potent corticosteroids and/or intralesional injections with triamcinolone acetonide. Beyond these therapies, patients may be started on courses of oral corticosteroid treatment. Those who are unresponsive to corticosteroids may be started on cyclosporine or mycophenolate mofetil. Other treatments that have been used in patient with LPP include antimalarials, retinoids, thalidomide, tetracycline, griseofulvin, IV methylprednisolone, topical calcineurin inhibitors, and topical minoxidil lotion (Assouly and Reygagne 2009).

Conclusion

Lichen planus is an immune-mediated disease that can affect individuals in different ways.

It may be limited to the skin or it may just affect nails, oral mucosa, or other mucosa. In some, it may involve a combination of these areas. Some may experience spontaneous resolution of the disease. For others, treatment depends on involvement of the disease.

Cross-References

- ▶ Alopecia Areata
- ▶ Immune Responses to the Hepatitis C Virus

References

Asch S, Goldenberg G. Systemic treatment of cutaneous lichen planus: an update. Cutis. 2011;87:129–34.
Assouly P, Reygagne P. Lichen planopilaris: update on diagnosis and treatment. Semin Cutan Med Surg. 2009;28:3–10.
Boyd AS, Neldner KH. Lichen planus. J Am Acad Dermatol. 1991;25:593–619.
Edwards PC, Kelsch R. Oral lichen planus: clinical presentation and management. J Can Dent Assoc. 2002;68:494–9.
Ellgehausen P, Elsner P, Burg G. Drug-Induced lichen planus. Clin Dermatol. 1998;16:325–32.
Ismail SB, Kumar SKS, Zain RB. Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis, management and malignant transformation. J Oral Sci. 2007;49:89–106.
Kang H, Alzolibani AA, Otberg N, Shapiro J. Lichen planopilaris. Dermatol Ther. 2008;21:249–56.
Lavanya N, Jayanthi P, Rao UK, Ranganathan K. Oral lichen planus: an update on pathogenesis and treatment. J Oral Maxillofac Pathol. 2011;15:127–32.
Le Cleach L, Chosidow ON. Clinical practice. Lichen planus. New Engl J Med. 2012;366:723–32.
Lukac J, Brozovic S, Vucicevuc-Boras V, Mravak-Stipetic M, Malenica B, Kusic Z. Serum autoantibodies to desmogleins 1 and 3 in patients with oral lichen planus. Croat Med J. 2006;47:53–8.
Piraccini BM, Saccani E, Starace M, Balestri R, Tosti A. Nail lichen planus: response to treatment and long term follow-up. Eur J Dermatol. 2010;20:489–96.
Rapini RP. Eczematous and papulosquamous diseases. In: Rapini RP, editor. Practical dermatopathology. Philadelphia: Elsevier Mosby; 2005. p. 45–59.
Roopashree MR, Gondhalekar RV, Shashikanth MC, George J, Thippeswamy SH, Shukla A. Pathogenesis of oral lichen planus- a review. J Oral Pathol Med. 2010;39:729–34.
Shengyuan L, Songpo Y, Wen W, Wenjing T, Haitao Z, Binyou W. Hepatitis C virus and lichen planus: a reciprocal association determined by a meta-analysis. Arch Dermatol. 2009;145:1040–7.
Shiohara T, Kano Y. Lichen planus and lichenoid dermatoses. In: Bolognia JL et al., editors. Dermatology. 2nd ed. Philadelphia: Mosby; 2008. p. 168.
Thompson DF, Skaehill PA. Drug-induced lichen planus. Pharmacotherapy. 1994;14:561–71.
Usatine RP, Tinitigan M. Diagnosis and treatment of lichen planus. Am Fam Physician. 2011;84:53–60.

Linear IgA Disease

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Synonyms

Adults: Adult linear IgA disease; IgA bullous pemphigoid; Linear dermatitis herpetiformis; Linear IgA bullous dermatosis; Linear IgA dermatitis herpetiformis; Linear IgA dermatosis; Linear IgA disease of adults; Pemphigoid linear IgA

Children: Benign chronic bullous dermatosis of childhood; Childhood linear IgA dermatitis herpetiformis; Chronic bullous disease of childhood; Chronic bullous disease of childhood; Juvenile dermatitis herpetiformis; Juvenile pemphigoid; Linear IgA disease of childhood

Definition

Linear IgA disease, which is a chronic subepidermal vesicular dermatosis of both children and adults, is defined by IgA autoantibodies to antigens in the BMZ (basement membrane zone). The two subgroups, chronic bullous disease of childhood and adult linear IgA disease, differ only in the age of onset.

While pathogenic IgA autoantibodies are occasionally observed in some of the other

blistering disorders in addition to the strong majority IgG type, IgA predominance is unique to this condition.

Numerous other conditions have been associated with linear IgA disease through varied and limited reports in the literature, which include a variety of other autoimmune disorders, gastrointestinal diseases, malignant conditions, and infections. The incidence of gluten-sensitive enteropathy, which is strongly associated with (immunopathologically similar) dermatitis herpetiformis (DH), ranges from 0 % to 24 % (Vassileva 1998; Labib et al. 1986; Hofmann et al. 2002; Guillaume et al. 1993). The incidence of other gastrointestinal disease is also much lower than that observed with DH. Many drugs have also been implicated in inducing linear IgA disease, most unequivocally vancomycin. This drug produces a toxic epidermal necrolysis-like presentation. The relevance of all these associations has yet to be determined.

Clinical

In children, annular erythema and blisters, which occur predominantly in flexural areas and the lower trunk, thigh, and groin, have been described as resembling a "crown of jewels."

Diagnosis may be difficult as the clinical and immunopathologic pattern may be similar to that of bullous pemphigoid (▶ Subepidermal Blistering Diseases: Bullous Pemphigoid) or dermatitis herpetiformis, with the latter being differentiated by a more granular pattern to BMZ IgA deposition.

The defining feature of this disorder, which is a linear deposition of IgA along the BMZ, is observed through direct IF and histopathologic examination. Inflammatory cells are observed in a subepidermal infiltrate particularly at the tips of dermal papillae that include T cells, eosinophils (but fewer than seen in bullous pemphigoid), and a predominance of neutrophils. Dermal capillary and papillary microabscesses are observed in some blisters, with older lesions showing more nonspecific findings.

Pathophysiology

Linear IgA disease is named for the pathogenic IgA antibodies, mostly monomeric IgA1, that target several different antigens in the adhesion complex at the BMZ (Egan et al. 1999; Leonard et al. 1984; Wojnarowska et al. 1994). Rarely, IgG antibodies are also detected in a small number of patients (Allen and Wojnarowska 2003; Kromminga et al. 2000). The primary target antigen is the 97 kDa shed ectodomain of BP180 (BPAG-2, type XVII collagen, or LAD1). Autoantibodies to other BP180 epitopes, such as the NC16A domain associated with bullous pemphigoid, are observed in some patients, as are antibodies to BP230 and the 285 kDa unique antigen LAD285 (Allen and Wojnarowska 2003; Ghohestani et al. 1997; Marinkovich et al. 1996; Wojnarowska et al. 1991; Zone et al. 1990; Georgi et al. 2001; Zillikens et al. 1999). Rarely, autoantibodies to anchoring fibril component collagen VII are detected but, unlike epidermolysis bullosa acquisita, are almost never associated with friction-induced mechanobullous scarring.

The deposition of IgA attracts other inflammatory cells such as T cells, eosinophils, and neutrophils, which in turn release additional cytokines and proteases that induce vesicle formation. Neutrophils play a prominent role in disease pathogenesis. If papillary microabscesses are present and numerous, the histopathologic findings in linear IgA disease are similar to those observed in dermatitis herpetiformis.

Direct IF shows the linear deposition of IgA along the BMZ and occasionally shows other immunoreactants such as IgG, IgM, and/or C3 (Leonard et al. 1982). Indirect IF detects circulating antibodies in a majority of patients, as opposed to with DH in which they are rarely found.

Cross-References

- ▶ Subepidermal Blistering Diseases: Bullous Pemphigoid

References

Allen J, Wojnarowska F. Linear IgA disease: the IgA and IgG response to the epidermal antigens demonstrates that intermolecular epitope spreading is associated with IgA rather than IgG antibodies, and is more common in adults. Br J Dermatol. 2003;149(5):977–85.

Egan CA, et al. IgA antibodies recognizing LABD97 are predominantly IgA1 subclass. Acta Derm Venereol. 1999;79(5):343–6.

Georgi M, et al. Mapping of epitopes on the BP180 ectodomain targeted by IgA and IgG autoantibodies in patients with the lamina lucida-type of linear IgA disease. Arch Dermatol Res. 2001;293(3):109–14.

Ghohestani RF, et al. Linear IgA bullous dermatosis with IgA antibodies exclusively directed against the 180- or 230-kDa epidermal antigens. J Invest Dermatol. 1997;108(6):854–8.

Guillaume JC, et al. Controlled trial of azathioprine and plasma exchange in addition to prednisolone in the treatment of bullous pemphigoid. Arch Dermatol. 1993;129(1):49–53.

Hofmann S, et al. Severity and phenotype of bullous pemphigoid relate to autoantibody profile against the NH2- and COOH-terminal regions of the BP180 ectodomain. J Invest Dermatol. 2002;119(5):1065–73.

Kromminga A, et al. Patients with bullous pemphigoid and linear IgA disease show a dual IgA and IgG autoimmune response to BP180. J Autoimmun. 2000;15(3):293–300.

Labib RS, et al. Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. J Immunol. 1986;136(4):1231–5.

Leonard JN, et al. Linear IgA disease in adults. Br J Dermatol. 1982;107(3):301–16.

Leonard JN, et al. Evidence that the IgA in patients with linear IgA disease is qualitatively different from that of patients with dermatitis herpetiformis. Br J Dermatol. 1984;110(3):315–21.

Marinkovich MP, et al. LAD-1, the linear IgA bullous dermatosis autoantigen, is a novel 120-kDa anchoring filament protein synthesized by epidermal cells. J Invest Dermatol. 1996;106(4):734–8.

Vassileva S. Drug-induced pemphigoid: bullous and cicatricial. Clin Dermatol. 1998;16(3):379–87.

Wojnarowska F, et al. Identification of the target antigen in chronic bullous disease of childhood and linear IgA disease of adults. Br J Dermatol. 1991;124(2):157–62.

Wojnarowska F, Bhogal BS, Black MM. Chronic bullous disease of childhood and linear IgA disease of adults are IgA1-mediated diseases. Br J Dermatol. 1994;131(2):201–4.

Zillikens D, et al. Autoantibodies in a subgroup of patients with linear IgA disease react with the NC16A domain of BP1801. J Invest Dermatol. 1999;113(6):947–53.

Zone JJ, et al. Identification of the cutaneous basement membrane zone antigen and isolation of antibody in linear immunoglobulin A bullous dermatosis. J Clin Invest. 1990;85(3):812–20.

Linker for Activation of T Cells (LAT)

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Synonyms

LAT

Definition

LAT is a transmembrane adaptor protein vital for TCR-mediated signaling. It is expressed primarily in T cells, but also in mast cells, NK cells, megakaryocytes, and pre-B cells.

Historical Background

Adaptive immune responses to pathogens are primarily mediated by helper T cells, which can stimulate macrophage phagocytosis, B cells class switching, and antibody secretion. Initial T cell activation is dependent on engagement of the T cell receptor (TCR) with the MHC-peptide complex on the surface of an antigen-presenting cell (APC). Upon ligation of the TCR, many proteins become highly tyrosine phosphorylated, including a 36–38-kDa protein that was known to localize in the plasma membrane. Because of the high degree of phosphorylation, it was hypothesized that this protein might play a critical role in linking TCR engagement to downstream signaling events in the cytoplasm. This protein, named *l*inker for *a*ctivation of *T* cells (LAT) was subsequently sequenced, revealing a very short extracellular domain, a transmembrane domain, and a long cytoplasmic tail with nine conserved tyrosine residues. Although LAT has no structural domains, it acts as a scaffolding protein, allowing for the binding of proteins to its multiple tyrosine motifs (Zhang et al. 1998). Deficiency of LAT in a human T cell line illustrated the importance of

LAT for TCR-mediated signaling events, such as ERK activation, calcium mobilization, and activation of the transcription factors AP-1 and NFAT (Finco et al. 1998; Zhang et al. 1999a). Additionally, LAT-deficient mice have a complete block in thymocyte development at the DN3 stage ($CD44^{-}CD25^{+}$), resulting in no mature T cells, thus demonstrating the crucial role of LAT-mediated signaling downstream of the pre-TCR complex (Zhang et al. 1999b). Recently, it was determined that LAT is also indispensible for the transition from DP ($CD4^{+}CD8^{+}$) to SP ($CD4^{+}$ or $CD8^{+}$) thymocytes (Shen et al. 2009).

The Role of LAT in T Cell Receptor Signaling

After TCR ligation with MHC-peptide, Lck, a Src family kinase, is activated and phosphorylates the cytoplasmic domains of TCRζ and CD3 at immunoreceptor tyrosine-based activation motifs (ITAMs). ZAP-70, a tyrosine kinase, can subsequently bind to these phosphorylated ITAMs, is activated by Lck, and then phosphorylates LAT. LAT, in turn, recruits Grb2, Gads, and PLC-γ1, all of which evoke disparate signaling cascades, vital for initiation of gene transcription (Samelson 2002). Among the tyrosine residues in LAT, the distal four are vital for T cell development, proliferation, and activation downstream of TCR engagement since mice with these tyrosine residues mutated resemble LAT-deficient mice (Sommers et al. 2001). In mice, LAT tyrosine 136 binds PLC-γ1, while tyrosines 175, 195, and 235 bind Grb2 and Gads (Zhang et al. 2000). LAT interaction with Grb2 recruits Son of Sevenless (SOS), a Ras guanine nucleotide exchange factor, to the LAT complex. SOS then initiates the Ras-Erk pathway, leading to activation of the transcription factor AP-1. Additionally, LAT recruits Gads, which is constitutively associated with the cytoplasmic adaptor molecule, SLP-76. SLP-76 associates with Vav, the guanine nucleotide exchange factor for Rac/Rho, which allows for actin polymerization and cytoskeleton rearrangement, important for T cell activation and division. Furthermore, Gads aids in PLC-γ1 recruitment and function. In addition to tethering PLC-γ1 to the LAT complex, Gads also indirectly recruits the Tec kinase, Itk, which phosphorylates and activates PLC-γ1. PLC-γ1 then functions to hydrolyze phosphatidylinositol bisphosphate (PIP_2) to inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 is essential to drive calcium flux and activation of the transcription factor, NFAT. Simultaneously, DAG mobilizes two downstream pathways. It recruits and allows for subsequent activation of protein kinase C (PKC) and additionally brings RasGRP1 to the plasma membrane. PKC activation ultimately causes NF-κB translocation to the nucleus. RasGRP1, a guanine nucleotide exchange factor similar to SOS, activates the Ras-Erk pathway (Roose et al. 2005). Collectively, these pathways downstream of LAT that are activated upon TCR stimulation are required for proper IL2 upregulation (Samelson 2002).

In addition to its essential role in the positive regulation of T cell activation, LAT can also recruit molecules to the signalosome which dampen signals emanating from the TCR. Gab2, the Grb2-associated binding protein 2, can bind Grb2 and Gads at steady state, and, when brought to the TCR synapse, it is phosphorylated by Zap-70. Gab2 phosphorylation creates a binding site for SHP2, a phosphatase which can dephosphorylate CD3ζ (Yamasaki et al. 2003). Not only does Grb2 recruit the negative regulator Gab2, but it can also bind SHIP-1, the SH2 domain-containing inositol polyphosphate 5-phosphatase-1. Dok-2, downstream of kinase 2, is anchored by SHIP-1 and acts to negatively regulate Zap-70 function (Dong et al. 2006). Therefore, through its binding partners, LAT is integral for proper calcium mobilization and MAPK activation as well as the prevention of prolonged positive signaling.

LAT in CD4 T Cell-Mediated Autoimmunity

LAT clearly plays an indispensable role in the positive regulation of thymocyte development as

LAT-deficient mice lack peripheral T cells. However, the disruption of LAT interaction with its binding partners results in T cell hyperproliferation in the periphery, demonstrating that LAT also plays a role in the negative regulation of T cell homeostasis. LATY136F knock-in mice, in which tyrosine 136 is replaced with a phenylalanine, contain a form of LAT unable to bind PLC-γ1. These mice have a block in thymocyte development; most thymocytes are unable to progress past the DN compartment due to insufficient signaling through the pre-TCR complex. Yet the T cells that do mature in the thymus and escape to the periphery are hyperproliferative, causing a fatal autoimmune-like disease. CD4 T cells dominate peripheral lymphoid organs and infiltrate other tissues, such as the liver, lungs, and kidneys. LATY136F CD4 T cells have an effector memory phenotype ($CD44^{hi}CD62L^{lo}$), express low levels of surface TCR, and secrete large amounts of T helper (Th)2 cytokines, such as IL-4. These mice have increased B cell, macrophage, and eosinophil numbers yet are devoid of CD8 T cells. High levels of serum IgE, IgG1, and IgM are detectable and serum autoantibodies are present in these mice (Aguado et al. 2002; Sommers et al. 2002). However, the autoimmune-like phenotype seen in LATY136F mice is not dependent on Th2 programming. LATY136F mice deficient in STAT6, a master regulator of Th2 development, display a similar CD4 T cell hyperproliferation when compared to LATY136F mice; however, the pathogenic cells are Th1 skewed, producing IFNγ and expressing elevated levels of T-bet (Archambaud et al. 2009).

The autoimmune-like phenotype seen in LATY136F mice suggests that the peripheral T cells could be responding to self-antigen presented on MHC molecules, inducing their proliferation. Indeed, a study uncovered that the thymic selection processes which are meant to prevent self-reactive T cells from entering the periphery are defective in LATY136F mice (Sommers et al. 2005). Furthermore, these mice do not contain T regulatory cells, which are normally critical to control T cell expansion (Koonpaew et al. 2006). Yet, there is also evidence that LATY136F T cell proliferation does not simply result from self-reactivity but from altered intrinsic mechanisms. First, LATY136F T cells are polyclonal and express low levels of surface TCR. Antigen, both self and foreign, is presented to CD4 T cells through MHC class II molecules on the surface of APCs. It was demonstrated that LATY136F T cells proliferate in MHC II-deficient hosts (Wang et al. 2008). Also, an inducible deletion model was developed, by which normal thymic development is allowed to occur through the use of a floxed wild-type LAT allele. This wild-type LAT is then deleted in mature T cells using a Cre-loxP system, allowing for expression of only the recessive mutant allele in peripheral T cells. These mice develop a disease similar to LATY136F mice, indicating that T cell hyperproliferation in the absence of LAT-PLC-γ1 interaction cannot be solely attributed to defective thymic selection (Chuck et al. 2010). Interestingly, mice with LAT inducibly deleted in mature T cells also developed a similar, yet less severe, autoimmune syndrome, characterized by CD4 T cell expansion and enhanced cytokine production (Mingueneau et al. 2009; Shen et al. 2010). Together, these data indicate that LAT functions as a negative regulator of T cell homeostasis and expansion. Specifically, in the absence of the LAT-PLC-γ1 interaction, CD4 T cells expand and produce Th2 cytokines, suggesting a fundamental role for LAT-PLC-γ1 signaling in the repression of this T helper cell commitment.

LAT in γδ T Cell-Mediated Autoimmunity

In addition to the knock-in mouse model with the LAT-PLC-γ1 binding site mutated, mice were generated in which LAT binding to both Gads and Grb2 was inhibited. These mice, LAT3YF mice, harbor mutations at Y175, Y195, and Y235. Whereas LATY136F mice have a partial block in development at the DN3 stage, LAT3YF mice have a complete block in the DN compartment, indicating that signaling through Gads and Grb2 is essential for pre-TCR signaling and thymocyte development of αβ T cells. γδ T cells, on the other hand, rearrange both TCR genes in the

DN compartment. LAT3YF mice contain decreased numbers of thymocytes with both TCR alleles rearranged and expressed on the cell surface; however, these cells are able to populate the periphery. By 6 months of age, the pool of γδ T cells in peripheral lymphoid organs expands, resulting in splenomegaly and lymphadenopathy. The hyperproliferative LAT3YF T cells produce Th2 cytokines, driving elevated serum IgE and IgG1 levels (Nunez-Cruz et al. 2003). These data indicate that LAT-mediated signaling through Gads and Grb2 is required for αβ T cell development and is also necessary to regulate γδ T cell homeostasis.

LAT-Mediated T Cell Proliferation

One aspect that is shared by all forms of LAT-mediated autoimmunity is the hyperproliferative nature of the T cells. Mutation of LAT binding sites results in a block in thymic development; therefore, T cells that do exit the thymus enter a lymphopenic environment. Lymphopenia-driven homeostatic proliferation of CD4 T cells occurs in two forms. The first proliferation is slow, dependent on IL7 and MHC interaction. Homeostatic proliferation can also be rapid, requiring antigen-MHC-driven TCR signaling. T cells with LAT deleted in the periphery fail to undergo rapid homeostatic proliferation (Shen et al. 2010). LATY136F T cells, which are unable to signal through LAT-PLCγ1, also do not undergo rapid proliferation upon transfer into T cell-deficient hosts but rather undergo slow proliferation. These T cells proliferate similar to wild-type T cells in an MHC-deficient environment but do not divide in the absence of IL7 (Wang et al. 2008). This indicates that IL7 plays an important role in T cell expansion in LAT mutant mice and that LAT is critical for the regulation of slow homeostatic proliferation.

Combined, the studies examining the different aspects of the LAT signalosome reveal novel requirements for this adaptor molecule during T cell development, activation, and homeostasis. Understanding both the positive and negative regulation that LAT exerts on T cells will improve our ability to develop potential drug targets to modulate T cell-mediated immune responses.

Cross-References

- ▶ NF-κB
- ▶ Nuclear Factor of Activated T Cells (NFAT)
- ▶ Rho/Rac GTPases
- ▶ SH2 Domain-containing Inositol Phosphatase-1 (SHIP)
- ▶ Tregs in the Liver

References

Aguado E, Richelme S, Nunez-Cruz S, Miazek A, Mura AM, Richelme M, Guo XJ, Sainty D, He HT, Malissen B, et al. Induction of T helper type 2 immunity by a point mutation in the LAT adaptor. Science. 2002;296:2036–40.

Archambaud C, Sansoni A, Mingueneau M, Devilard E, Delsol G, Malissen B, Malissen M. STAT6 deletion converts the Th2 inflammatory pathology afflicting Lat(Y136F) mice into a lymphoproliferative disorder involving Th1 and CD8 effector T cells. J Immunol. 2009;182:2680–9.

Chuck MI, Zhu M, Shen S, Zhang W. The role of the LAT-PLC-gamma1 interaction in T regulatory cell function. J Immunol. 2010;184:2476–86.

Dong S, Corre B, Foulon E, Dufour E, Veillette A, Acuto O, Michel F. T cell receptor for antigen induces linker for activation of T cell-dependent activation of a negative signaling complex involving Dok-2, SHIP-1, and Grb-2. J Exp Med. 2006;203:2509–18.

Finco TS, Kadlecek T, Zhang W, Samelson LE, Weiss A. LAT is required for TCR-mediated activation of PLCgamma1 and the Ras pathway. Immunity. 1998;9:617–26.

Koonpaew S, Shen S, Flowers L, Zhang W. LAT-mediated signaling in CD4+CD25+ regulatory T cell development. J Exp Med. 2006;203:119–29.

Mingueneau M, Roncagalli R, Gregoire C, Kissenpfennig A, Miazek A, Archambaud C, Wang Y, Perrin P, Bertosio E, Sansoni A, et al. Loss of the LAT adaptor converts antigen-responsive T cells into pathogenic effectors that function independently of the T cell receptor. Immunity. 2009;31:197–208.

Nunez-Cruz S, Aguado E, Richelme S, Chetaille B, Mura AM, Richelme M, Pouyet L, Jouvin-Marche E, Xerri L, Malissen B, et al. LAT regulates gammadelta T cell homeostasis and differentiation. Nat Immunol. 2003;4:999–1008.

Roose JP, Mollenauer M, Gupta VA, Stone J, Weiss A. A diacylglycerol-protein kinase C-RasGRP1 pathway

directs Ras activation upon antigen receptor stimulation of T cells. Mol Cell Biol. 2005;25:4426–41.
Samelson LE. Signal transduction mediated by the T cell antigen receptor: the role of adapter proteins. Annu Rev Immunol. 2002;20:371–94.
Shen S, Zhu M, Lau J, Chuck M, Zhang W. The essential role of LAT in thymocyte development during transition from the double-positive to single-positive stage. J Immunol. 2009;182:5596–604.
Shen S, Chuck MI, Zhu M, Fuller DM, Yang CW, Zhang W. The importance of LAT in the activation, homeostasis, and regulatory function of T cells. J Biol Chem. 2010;285:35393–405.
Sommers CL, Menon RK, Grinberg A, Zhang W, Samelson LE, Love PE. Knock-in mutation of the distal four tyrosines of linker for activation of T cells blocks murine T cell development. J Exp Med. 2001;194:135–42.
Sommers CL, Park CS, Lee J, Feng C, Fuller CL, Grinberg A, Hildebrand JA, Lacana E, Menon RK, Shores EW, et al. A LAT mutation that inhibits T cell development yet induces lymphoproliferation. Science. 2002;296:2040–3.
Sommers CL, Lee J, Steiner KL, Gurson JM, Depersis CL, El-Khoury D, Fuller CL, Shores EW, Love PE, Samelson LE. Mutation of the phospholipase C-gamma1-binding site of LAT affects both positive and negative thymocyte selection. J Exp Med. 2005;201:1125–34.
Wang Y, Kissenpfennig A, Mingueneau M, Richelme S, Perrin P, Chevrier S, Genton C, Lucas B, DiSanto JP, Acha-Orbea H, et al. Th2 lymphoproliferative disorder of LatY136F mutant mice unfolds independently of TCR-MHC engagement and is insensitive to the action of Foxp3+ regulatory T cells. J Immunol. 2008;180:1565–75.
Yamasaki S, Nishida K, Sakuma M, Berry D, McGlade CJ, Hirano T, Saito T. Gads/Grb2-mediated association with LAT is critical for the inhibitory function of Gab2 in T cells. Mol Cell Biol. 2003;23:2515–29.
Zhang W, Sloan-Lancaster J, Kitchen J, Trible RP, Samelson LE. LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. Cell. 1998;92:83–92.
Zhang W, Irvin BJ, Trible RP, Abraham RT, Samelson LE. Functional analysis of LAT in TCR-mediated signaling pathways using a LAT-deficient Jurkat cell line. Int Immunol. 1999a;11:943–50.
Zhang W, Sommers CL, Burshtyn DN, Stebbins CC, DeJarnette JB, Trible RP, Grinberg A, Tsay HC, Jacobs HM, Kessler CM, et al. Essential role of LAT in T cell development. Immunity. 1999b;10:323–32.
Zhang W, Trible RP, Zhu M, Liu SK, McGlade CJ, Samelson LE. Association of Grb2, Gads, and phospholipase C-gamma 1 with phosphorylated LAT tyrosine residues. Effect of LAT tyrosine mutations on T cell angigen receptor-mediated signaling. J Biol Chem. 2000;275:23355–61.

Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance

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Synonyms

Immune function of liver sinusoidal endothelial cells

Definition

Liver sinusoidal endothelial cells (LSECs) constitute a unique population of microvascular endothelial cells that line the hepatic sinusoids and posses potent scavenger activity. LSECs maintain a very important role in detecting the presence of pathogen-associated molecular patterns (PAMPs) and initiate inflammation while being able to control deleterious inflammation in response to continuous physiological exposure toward PAMPs contained in portal venous blood. Importantly, LSEC can present and cross-present antigen to both CD8+ as well as CD4+ T cells. T cell priming by antigen-presenting LSEC leads to unresponsiveness or regulatory T cell development. Thus, LSEC appears to contribute to the unique tolerogenic function of the liver that is best exemplified by the propensity of liver grafts to be accepted in the absence of immunosuppression.

Introduction

The liver receives blood through the hepatic artery and the portal vein that is draining the venous nutrient-rich blood from the

gastrointestinal tract. After extensive ramification branches of the hepatic artery and the portal vein merge in the hepatic sinusoids giving rise to a mixed arterio-venous blood supply for the sinusoidal cell populations, i.e., Kupffer cells, liver sinusoidal endothelial cells (LSECs), stellate cells, liver-associated lymphocytes, and hepatocytes (Thomson and Knolle 2010). This results in a low oxygen-tension in the blood flowing through hepatic sinusoids and the absence of typical arterial and venous branches in the hepatic microcirculation (▶ Liver Vasculature and Microvasculature). Due to the sudden increase in the cumulative vascular diameter in the sinusoidal circulation of the liver, there is a drop in perfusion pressure that leads to a slow and chaotic blood flow within hepatic sinusoids. This allows circulating immune cells to establish contact with liver sinusoidal cells without the help of selectins that normally slow down blood-borne immune cells to allow for interaction between adhesion molecules (Wong et al. 1997) (▶ Adaptive Immune Cells in the Liver).

Liver sinusoidal endothelial cells (LSECs) constitute a unique population of microvascular endothelial cells that are very thin (150–200 nm) and line the hepatic sinusoids. Behind LSEC is located another liver-resident cell population, the stellate cells. The space between LSEC/stellate cells and hepatocytes is called the space of Dissé. Liver sinusoids are devoid of a basement membrane, and fenestrae in LSEC with a mean diameter of 50–200 nm allow for diffusion of blood-borne molecules with a size smaller than 30 nm into the space of Dissé (Kempka and Kolb-Bachofen 1988) where they can interact with the microvilli of hepatocytes (▶ Ultrastructure of the Liver Sinusoid). The size of fenestrae in LSEC is dynamically regulated by the actin cytoskeleton and decreases as a consequence of exposure to toxins such as alcohol. Following long-standing toxin exposure, vascular remodeling with loss of fenestrae and development of a basement membrane may occur and is considered an ultrastructural determinant of liver cirrhosis. The size and numbers of fenestrae also decrease with age. The hepatic blood flow is regulated through the contractile activity of the perisinusoidal cells, i.e., stellate cells, that control the sinusoidal diameter. As stellate cells are relatively abundant and ubiquitously present in hepatic sinusoids, their function in regulating hepatic blood flow has been difficult to study.

LSECs have an important function in maintaining sinusoidal blood flow by producing plasminogen activator inhibitor to prevent inadvertent coagulation in liver sinusoids due to the unusual conditions of blood flow. Also, LSEC contributes to liver regeneration and hepatocyte proliferation through the release of hepatocyte growth factor (Ding et al. 2010). Although LSECs are a liver-resident cell population, they can also repopulate from circulating bone-marrow-derived endothelial precursors that produce even larger amounts of hepatocyte growth factor compared to liver-resident LSEC (Wang et al. 2012), thus increasing liver regeneration during situations where substantial losses of liver tissue have occurred.

Thus, LSECs are a highly specialized population of microvascular endothelial cells optimally suited to fit the functional demands of the hepatic microvasculature: They separate circulating immune cells from hepatocytes while allowing for proper execution of the metabolic function of hepatocytes.

Scavenger Function

LSECs are distinct from other microvascular endothelial cells by their extraordinary endocytic function that is conserved in all vertebrates. This scavenger function has for long been underestimated because Kupffer cells were considered to be the principal scavenger cell population of the liver (KC and Tolerance). More sophisticated methods of investigation, however, have revealed that LSECs but not Kupffer cells are responsible for clearance of molecules smaller than 200 nm by receptor-mediated endocytosis from the circulation (Smedsrod 2004). The scavenger function in LSEC is supported by very rapid turnover rates for receptor-mediated endocytosis, delivery of the ligand cargo into an endo(lyso)somal compartment, separation from

the receptor, and receptor reshuttling to the cell surface that all occurs within 15 s (Knolle and Gerken 2000). The molecular mechanisms allowing for this rapid receptor turnover have not been characterized yet. The combination of slow blood flow together with LSEC scavenger function synergizes for most efficient clearance of liver sinusoidal blood from waste products or other molecules.

LSECs employ several receptors for their scavenger function that allow for uptake of a broad range of ligands. These receptors comprise the mannose receptor, the collagen-alpha chain receptor, the scavenger receptor, and the Fc-gamma receptor. The scavenger receptor is involved in most efficient uptake of extracellular matrix molecules such as hyaluronan, chondroitin sulfate, type I and III collagens as well as oxidized LDL, advanced glycation end products, and coagulation products. The mannose receptor similarly supports efficient uptake of denatured collagen but also of lysosomal enzymes for endolysosomal degradation of endocytosed degradation/waste products within LSEC that produce little if any of these lysosomal enzymes themselves (Malovic et al. 2007; Smedsrod 2004). It is of interest to note that LSECs metabolize their endocytosed ligands into acetate and lactate, that in turn serve as metabolites for anaerobic energy generation via glycolysis in other sinusoidal cell populations or hepatocytes (Smedsrod 2004). Therefore, LSEC's scavenger function may operate to provide sufficient fuel for energy production under low oxygen-tension in addition to the obvious clearance function to eliminate waste or degraded molecules from the blood circulation. The scavenger function of LSEC contributes to the well-known first-pass effect of the liver and is a key determinant of hepatic recycling of extracellular matrix molecules.

The physiological scavenger function may be exploited by hepatotropic pathogens that target the liver to exit the blood stream and subsequently infect their target cells, i.e., hepatocytes (Protzer et al. 2012). Duck Hepatitis B Virus, which serves as a experimental model system for Hepatitis B Virus infection, is first taken up by LSEC before hepatocytes presumably following transcytosis (Breiner et al. 2001). Similar observations were made for infection with Hepatitis C Virus (Pohlmann et al. 2003) (► Immune Responses to the Hepatitis C Virus) and for adenovirus (Ganesan et al. 2011). The sinusoidal cells in the liver seem to provide a physical barrier for pathogens even for pathogens that can migrate such as plasmodium sporozoites, because these pathogens target Kupffer cells before eventually infecting hepatocytes (Pradel and Frevert 2001). Thus, LSEC's scavenger function may be exploited by viruses to exit the blood stream and infect their target hepatocytes in a second step following transcytosis.

Innate Immune Function

Expression of immune sensory molecules, such as Toll-like receptors (TLRs), is not restricted to myeloid immune cells but is also found in nonimmune cells (both parenchymal and nonparenchymal cells) in various peripheral organs. In the liver, LSECs are strategically positioned to scavenge gut-derived bacterial degradations products from portal venous blood (van Oosten et al. 2001). They express several immune sensory receptors at the cell surface, in endosomal compartments, and within the cytosol. Agonistic activation through TLR1 to 4, 6, 8, or 9 at the cell surface or in endosomal compartments induces functional activation in LSEC, leading to expression of pro-inflammatory mediators such as IL-6 or TNF (Martin-Armas et al. 2006; Wu et al. 2010). Activation with agonistic ligands to cytosolic sensory receptors such as RIG-I or MDA5 also leads to expression of pro-inflammatory IL-6 (Kern et al. 2010). It is of interest to note that LSECs are most sensitive to TLR stimulation and require only very small concentrations of TLR ligands for induction of pro-inflammatory cytokine expression (Knolle et al. 1997). This sensitivity may be related to the extraordinary endocytic scavenger function of LSEC, because many TLRs are preferentially located in endosomal compartment or only then start their

signaling activity once they reach this compartment. Thus, LSECs combine extraordinary scavenger function with innate immune sensory function, which attributes a particular sentinel function to this cell population in detecting infectious microorganisms or their degradation products.

The preferential infection of LSEC before hepatocytes and the expression of pro-inflammatory mediators are responsible for the rapid induction of the liver acute phase response. Hepatocytes respond to IL-6 that is released from LSEC but also Kupffer cells with expression of acute phase proteins like C-reactive protein and increased expression of coagulation and complement factors (Baumann and Gauldie 1994; Fey and Gauldie 1990). These factors serve to increase the systemic responsiveness of the immune system against infectious microorganisms and also provide the basis for an increased capacity for repair and regeneration of damage inflicted by the immune response.

The sentinel function of LSEC and also Kupffer cells raises the question why the continuous exposure of the liver to gut-derived bacterial degradation products carried out by portal venous blood does not lead to local inflammation in the liver. As scavenger function of LSEC is closely linked to their sentinel function, it is unlikely that LSECs are unresponsive under physiological conditions in the absence of local inflammation. A number of mechanisms have been proposed to limit local inflammation in the liver. Tachyphylaxis and adaptation of LSEC as well as Kupffer cells toward continuous low dose exposure toward pathogen-associated molecular patterns (PAMPs) may limit the expression of pro-inflammatory mediators in these cells while at the same time conserving the responsiveness toward increased concentrations of PAMPs that indicate infection by microorganisms. The sensing of relative increases rather than absolute concentrations of ligands by immune sensory receptors is operative in other sensory systems and provides the most likely explanation for the absence of local inflammation under physiological situations (Knolle et al. 1997). Together with induction of pro-inflammatory mediators, LSECs also show increased expression of anti-inflammatory substances such as prostaglandin that may act in an autocrine negative feedback loop to limit local inflammation (Rieder et al. 1990). Similarly, IL-10 released from Kupffer cells controls expression of pro-inflammatory mediators by LSEC. Thus, cell-intrinsic as well as paracrine mechanisms limit responsiveness of immune sensory receptors in LSEC and thereby seem to prevent induction of hepatic inflammation. It is likely that the high regenerative capacity of the liver conceals the actual extent of immune-mediated damage during inflammation. As expression of hepatocyte growth factor is driven by activation of LSEC, it appears that both immune-mediated damage and repair of injured hepatocytes are orchestrated by liver sinusoidal cells.

Antigen-Presenting Cell Function

The extraordinary scavenger function also forms the basis for another important immune function of LSEC, i.e., antigen presentation. Presentation of peptides derived from endogenous antigens occurs mainly in the context of MHC class I molecules and only to a much lesser extent in the context of MHC class II molecules (Kurts et al. 2010). Peptides associated with MHC class II molecules are mostly derived from proteins that were taken up by receptor-mediated endocytosis or macro-endocytosis. However, peptides derived from exogenous antigens taken up by receptor-mediated endocytosis can also associate with MHC class I molecules in some specialized cell types, a process that has been named cross-presentation (Kurts et al. 2010). Intensive research over the past years has demonstrated that cross-presented antigens are endocytosed through defined receptors and transported into key endosomal compartments competent for executing cross-presentation. Both the mannose receptor and another C-type lectin, DNGR1, have been shown to shuttle antigen for cross-presentation into early recycling

endosomes. These recycling endosomes contain also the necessary molecular machinery required to export antigens into the cytosol where they are degraded by the proteasome, re-import peptides into the endosome, and load these onto MHC class I molecules present in this compartment (Kurts et al. 2010; Osorio and Reis e Sousa 2011). This knowledge on the molecular mechanisms of cross-presentation is crucial for further development of novel vaccine strategies to improve existing prophylactic vaccination schemes and to design more effective therapeutic vaccination protocols.

Cross-Presentation by LSEC to CD8 T cells

Similar to dendritic cells and macrophages, LSECs bear the capacity to cross-present exogenous antigens to CD8 T cells (Limmer et al. 2000). Key to efficient cross-presentation in LSEC is receptor-mediated endocytosis. Direct comparison of cross-presentation following intravenous antigen application revealed that LSECs were faster and more efficient than $CD8^+$ splenic dendritic cells. While dendritic cells retained the antigen for longer time periods resulting in prolonged cross-presentation, LSEC rapidly eliminated endocytosed antigen, leading to shorter lived cross-presentation ability (Schurich et al. 2009). The molecular mechanisms determining cross-presentation in LSEC, however, remain unclear. Antigen routing seems critical for cross-presentation in LSEC as antibody-conjugated antigens endocytosed via the Fc-gamma receptor are not cross-presented on MHC class I molecules (Schurich et al. 2009). In contrast, the mannose receptor, critical for cross-presentation of ovalbumin by dendritic cells, is dispensable for cross-presentation by LSEC (Schurich et al. 2009), indicating that other receptors or other molecular mechanisms awaiting identification facilitate cross-presentation in this liver-resident cell population.

There are fundamental differences between dendritic cells and LSEC in cross-presentation. LSECs do not require a licensing step in order to stimulate naïve CD8 T cells whereas dendritic cells need to be stimulated by CD4 T helper cells or NKT cells (Kurts et al. 2010) (Semmling et al. 2010). This suggests that LSECs employ different means to initiate activation of naïve CD8 T cells compared to professional antigen-presenting cells such as dendritic cells.

It is known that the outcome of stimulation of naïve CD8 T cells by dendritic cells depends on the maturation status of the professional antigen-presenting cell. Activation of dendritic cells by innate immune stimuli or help by CD4 T helper/NKT cells initiates changes in the expression of co-stimulatory molecules that facilitates differentiation of naïve T cells into effector or memory T cells, a process that has been termed cross-priming. In contrast, lack of such stimulation of antigen-presenting dendritic cells results in insufficient stimulation of naïve T cells that eventually leads to their clonal deletion, which has been termed cross-tolerance (Kurts et al. 1997). Cross-presentation by LSEC leads to a third outcome of naïve T cell stimulation that is characterized by initial clonal expansion associated with a lack of responsiveness toward T cell receptor signaling but not clonal deletion. This particular T cell development critically depends on co-inhibitory signaling through B7H1 expressed on LSEC and its ligand PD1 expressed on T cells (Diehl et al. 2008). As T cells only express PD1 upon primary activation and LSECs upregulate B7H1 expression upon cognate MHC-restricted interaction with T cells, the differentiation of T cells by cross-presenting LSEC is a dynamic process occurring over 12–24 h (Schurich et al. 2010). Due to the lack of restimulation by signals delivered through the T cell receptor, $CD8^+$ T cells activated by LSEC failed to kill antigen-expressing tumor cells in the absence of co-stimulatory signals, suggesting that $CD8^+$ T cells became tolerant after antigen-specific stimulation by LSEC (Limmer et al. 2000; Limmer et al. 2005). It is tempting to speculate that the prominent scavenger function of LSEC together with their capacity to cross-present soluble circulating antigen to naïve CD8 T cells skews T cell responses to non-responsiveness toward these antigens, similar to the observation that primary activation of T cells by antigen-expressing hepatocytes (▶ Primary T-Cell Activation in Liver) led to subsequent

lack of T cell activity against the same antigen presented at other sites (Bowen et al. 2004).

Recently, it was found that cross-presentation of hepatocyte-derived antigens elicits a non-canonical effector function of activated $CD8^+$ T cells (▶ Primary T-Cell Activation in Liver). Such LSEC cross-presentation of antigens derived from virus-infected hepatocytes triggered expression of TNF by activated effector $CD8^+$ T cells, which then acted specifically on virus-infected hepatocytes to initiate caspase-dependent apoptotic cell death (Wohlleber et al. 2012). Direct interaction of activated $CD8^+$ T cells with virus-infected hepatocytes accounted only for 50 % of total cytotoxic T cell activity during viral hepatitis, revealing that T cells access hepatocytes even across the sinusoidal barrier (Warren et al. 2006) but is complemented by the non-canonical T cell effector function initiated by cross-presenting LSEC (▶ Ultrastructure of the Liver Sinusoid).

MHC Class II–Restricted Antigen Presentation of LSEC to CD4 T cells

LSEC can also initiate MHC class II–restricted antigen presentation to CD4 T cells. However, MHC class II–restricted presentation requires higher concentrations of antigen compared to cross-presentation on MHC class I molecules (Knolle et al. 1999; Lohse et al. 1996). Interestingly, LSEC can also prime naïve CD4 T cells, indicating that they function as semiprofessional antigen-presenting cells (Knolle et al. 1999). However, the outcome of MHC class II–restricted antigen presentation by LSEC is distinct from that by professional antigen-presenting cells such as dendritic cells. Naïve CD4 T cells primed by LSEC do not differentiate into conventional T helper cells (Knolle et al. 1999) but rather differentiate into T cells with regulatory function (Kruse et al. 2009). Interestingly, regulatory T cells induced by antigen-presenting LSEC express low levels of Foxp3 (Kruse et al. 2009), which re-iterates the notion that T cell priming by LSEC is dominated by molecular mechanisms that are distinct from those acting in immunogenic or tolerogenic dendritic cells. Antigen presentation by LSEC does not promote immunity but rather contributes to suppression of T cells with indirect allo-specificity in a transplantation setting (Banshodani et al. 2013; Tokita et al. 2006), which indicates a potential protective role of LSEC during liver transplantation where only macrovascular endothelial cells but not LSEC are replaced by recipient endothelial cells (Gao et al. 2001).

A close interaction between the gut and the liver has been proposed to exist and to contribute to liver pathology (Adams and Eksteen 2006). It was shown that the chemokine CCL25 produced by LSEC recruits CD4 T cells with the cognate receptor CCR9 that were generated in the gut to the liver (Eksteen et al. 2004). Autoreactive pathogenic $CCR9^+$ $CD4^+$ T cells contribute to inflammatory bowel disease and the same $CCR9^+$ T cells in the liver may cause sclerosing cholangitis (Eksteen et al. 2004), providing a mechanistic explanation for the clinical association of inflammatory bowel disease and primary sclerosing cholangitis. Along this line, it was also demonstrated that antigen-presenting LSEC can generate $CCR9^+$ $CD4^+$ T cells that home to the gut (Neumann et al. 2012), thereby demonstrating the mutual interaction between gut and liver in induction of an enterohepatic circulation of CCR9 expressing T cells (▶ Ultrastructure of the Liver Sinusoid).

CD4 T cells are also actively recruited in an antigen-independent fashion through LSECs that express particular chemokines. As explained above, CCL25 recruits pathogenic CCR9+ T cells to the liver (Eksteen et al. 2004). Furthermore, regulatory T cells adhered in a CCR4-dependent fashion to LSEC and were preferentially recruited to inflamed liver tissue by CXCR3-dependent arrest on inflammatory dendritic cells in the liver (Oo et al. 2010). However, pro-inflammatory Th17 cells were also recruited by LSEC to the inflamed liver by a chemokine-dependent process (Oo et al. 2012). The transmigration of T cells into the parenchyma is also facilitated by chemokines that are transcytosed by LSEC to the apical phase (Schrage et al. 2008) and is modulated by the interaction of LSEC with hepatocytes (Edwards et al. 2005). Thus, there is a complex interaction

of LSEC with circulating CD4 T cells that appears to contribute to development as well as control of local immune responses in the liver.

Sinusoidal Cell Cross-Talk

Beyond antigen-specific interaction, LSECs also interact with other cell populations and influence their functional behavior. Interaction with LSEC inhibits the function of dendritic cells to initiate T cell priming (Schildberg et al. 2008), indicating that dendritic cells fail to function as antigen-presenting cells in vivo as long as they remain in the liver in physical contact with LSEC but regain their function to initiate T cell response upon migration into the draining lymph node (Bertolino 2008). There is also evidence that C-type lectins such as LSECTin and the mannose receptor expressed on LSEC directly influence the effector function of activated $CD4^+$ T cells, thereby attenuating T cell immunity (Tang et al. 2009) and promoting local tumor immune escape in the liver (Arteta et al. 2010). LSEC even inhibits inflammatory $CD4^+$ T cell activity through IL-10-dependent induction of B7H1 and co-inhibitory signaling through PD1 on the T cell side (Carambia et al. 2013). Taken together, LSECs also contribute to local immune regulation of T cell immunity in the liver by antigen-independent interaction with T cells and also dendritic cells.

Conclusion

Liver sinusoidal endothelial cells (LSECs) line the hepatic sinusoids, the microvessels of the liver, and delineate the space of Dissé between hepatocytes and circulating cells in sinusoidal blood. LSECs bear extraordinary scavenger activity to eliminate waste products and degraded extracellular matrix molecules from the sinusoidal blood stream. They also contribute to elimination of bacterial degradation products derived from the gastrointestinal tract. LSECs express pattern recognition receptors and bear sentinel activity to detect the presence of PAMPs and to initiate inflammation, but several mechanisms control deleterious inflammation in response to continuous physiological exposure toward PAMPs contained in portal venous blood. Importantly, LSECs function as organ-resident antigen-presenting cells to stimulate CD8+ as well as CD4+ T cells. The outcome of T cell priming by antigen-presenting LSEC is the development of T cells that rather have regulatory function or are non-responsive to T cell receptor-mediated stimulation. Together with antigen-independent mechanisms that control T cell effector functions, LSECs appear to contribute to the unique tolerogenic function of the liver that is best exemplified by the propensity of liver grafts to be accepted in the absence of immunosuppression.

References

Adams DH, Eksteen B. Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease. Nat Rev Immunol. 2006;6:244–51.

Arteta B, Lasuen N, Lopategi A, Sveinbjornsson B, Smedsrod B, Vidal-Vanaclocha F. Colon carcinoma cell interaction with liver sinusoidal endothelium inhibits organ-specific antitumor immunity through interleukin-1-induced mannose receptor in mice. Hepatology. 2010;51:2172–82.

Banshodani M, Onoe T, Shishida M, Tahara H, Hashimoto S, Igarashi Y, Tanaka Y, Ohdan H. Adoptive transfer of allogeneic liver sinusoidal endothelial cells specifically inhibits T cell responses to cognate stimuli. Cell Transplant. 2013;22(9):1695–708. doi:10.3727/096368912X657738. Epub 2012 Oct 8.

Baumann H, Gauldie J. The acute phase response. Immunol Today. 1994;15:74–80.

Bertolino P. Impaired function of dendritic cells translocating the liver sinusoids: a veto effect contributing to intrahepatic tolerance? Euro J Immunol. 2008;38:938–41.

Bowen DG, Zen M, Holz L, Davis T, McCaughan GW, Bertolino P. The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. J Clin Invest. 2004;114:701–12.

Breiner KM, Schaller H, Knolle PA. Endothelial cell-mediated uptake of a hepatitis B virus: a new concept of liver targeting of hepatotropic microorganisms. Hepatology. 2001;34:803–8.

Carambia A, Frenzel C, Bruns OT, Schwinge D, Reimer R, Hohenberg H, Huber S, Tiegs G, Schramm C, Lohse AW, et al. Inhibition of inflammatory CD4 T cell activity by murine liver sinusoidal endothelial cells. J Hepatol. 2013;58:112–8.

Diehl L, Schurich A, Grochtmann R, Hegenbarth S, Chen L, Knolle PA. Tolerogenic maturation of liver

sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8+ T cell tolerance. Hepatology. 2008;47:296–305.

Ding BS, Nolan DJ, Butler JM, James D, Babazadeh AO, Rosenwaks Z, Mittal V, Kobayashi H, Shido K, Lyden D, et al. Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. Nature. 2010;468:310–5.

Edwards S, Lalor PF, Nash GB, Rainger GE, Adams DH. Lymphocyte traffic through sinusoidal endothelial cells is regulated by hepatocytes. Hepatology. 2005;41:451–9.

Eksteen B, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hubscher SG, Briskin M, Salmon M, Adams DH. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. J Exp Med. 2004;200:1511–7.

Fey GH, Gauldie J. The acute phase response of the liver in inflammation. Prog Liver Dis. 1990;9:89–116.

Ganesan LP, Mohanty S, Kim J, Clark KR, Robinson JM, Anderson CL. Rapid and efficient clearance of blood-borne virus by liver sinusoidal endothelium. PLoS Pathog. 2011;7:e1002281.

Gao Z, McAlister VC, Williams GM. Repopulation of liver endothelium by bone-marrow-derived cells. Lancet. 2001;357:932–3.

Kempka G, Kolb-Bachofen V. Binding, uptake, and transcytosis of ligands for mannose-specific receptors in rat liver: an electron microscopic study. Exp Cell Res. 1988;176:38–48.

Kern M, Popov A, Scholz K, Schumak B, Djandji D, Limmer A, Eggle D, Sacher T, Zawatzky R, Holtappels R, et al. Virally infected mouse liver endothelial cells trigger CD8+ T-cell immunity. Gastroenterology. 2010;138:336–46.

Knolle PA, Gerken G. Local control of the immune response in the liver. Immunol Rev. 2000;174:21–34.

Knolle PA, Loser E, Protzer U, Duchmann R, Schmitt E, Zum Buschenfelde KH, Rose-John S, Gerken G. Regulation of endotoxin-induced IL-6 production in liver sinusoidal endothelial cells and Kupffer cells by IL-10. Clin Exp Immunol. 1997;107:555–61.

Knolle PA, Schmitt E, Jin S, Germann T, Duchmann R, Hegenbarth S, Gerken G, Lohse AW. Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. Gastroenterology. 1999;116:1428–40.

Kruse N, Neumann K, Schrage A, Derkow K, Schott E, Erben U, Kuhl A, Loddenkemper C, Zeitz M, Hamann A, et al. Priming of CD4+ T cells by liver sinusoidal endothelial cells induces CD25low forkhead box protein 3- regulatory T cells suppressing autoimmune hepatitis. Hepatology. 2009;50:1904–13.

Kurts C, Kosaka H, Carbone FR, Miller JF, Heath WR. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. J Exp Med. 1997;186:239–45.

Kurts C, Robinson BW, Knolle PA. Cross-priming in health and disease. Nat Rev Immunol. 2010;10:403–14.

Limmer A, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med. 2000;6:1348–54.

Limmer A, Ohl J, Wingender G, Berg M, Jungerkes F, Schumak B, Djandji D, Scholz K, Klevenz A, Hegenbarth S, et al. Cross-presentation of oral antigens by liver sinusoidal endothelial cells leads to CD8 T cell tolerance. Eur J Immunol. 2005;35:2970–81.

Lohse AW, Knolle PA, Bilo K, Uhrig A, Waldmann C, Ibe M, Schmitt E, Gerken G, Meyer Zum Buschenfelde KH. Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells. Gastroenterology. 1996;110:1175–81.

Malovic I, Sorensen KK, Elvevold KH, Nedredal GI, Paulsen S, Erofeev AV, Smedsrod BH, McCourt PA. The mannose receptor on murine liver sinusoidal endothelial cells is the main denatured collagen clearance receptor. Hepatology. 2007;45:1454–61.

Martin-Armas M, Simon-Santamaria J, Pettersen I, Moens U, Smedsrod B, Sveinbjornsson B. Toll-like receptor 9 (TLR9) is present in murine liver sinusoidal endothelial cells (LSECs) and mediates the effect of CpG-oligonucleotides. J Hepatol. 2006;44:939–46.

Neumann K, Kruse N, Szilagyi B, Erben U, Rudolph C, Flach A, Zeitz M, Hamann A, Klugewitz K. Connecting liver and gut: murine liver sinusoidal endothelium induces gut tropism of CD4+ T cells via retinoic acid. Hepatology. 2012;55:1976–84.

Oo YH, Banz V, Kavanagh D, Liaskou E, Withers DR, Humphreys E, Reynolds GM, Lee-Turner L, Kalia N, Hubscher SG, et al. CXCR3-dependent recruitment and CCR6-mediated positioning of Th-17 cells in the inflamed liver. J Hepatol. 2012;57:1044–51.

Oo YH, Weston CJ, Lalor PF, Curbishley SM, Withers DR, Reynolds GM, Shetty S, Harki J, Shaw JC, Eksteen B, et al. Distinct roles for CCR4 and CXCR3 in the recruitment and positioning of regulatory T cells in the inflamed human liver. J Immunol. 2010;184:2886–98.

Osorio F, Reis e Sousa C. Myeloid C-type lectin receptors in pathogen recognition and host defense. Immunity. 2011;34:651–64.

Pohlmann S, Zhang J, Baribaud F, Chen Z, Leslie GJ, Lin G, Granelli-Piperno A, Doms RW, Rice CM, McKeating JA. Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR. J Virol. 2003;77:4070–80.

Pradel G, Frevert U. Malaria sporozoites actively enter and pass through rat Kupffer cells prior to hepatocyte invasion. Hepatology. 2001;33:1154–65.

Protzer U, Maini MK, Knolle PA. Living in the liver: hepatic infections. Nat Rev Immunol. 2012;12:201–13.

Rieder H, Ramadori G, Allmann KH, Meyer ZBK. Prostanoid release of cultured liver sinusoidal

endothelial cells in response to endotoxin and tumor necrosis factor. Comparison with umbilical vein endothelial cells. J Hepatol. 1990;11:359–66.
Schildberg FA, Hegenbarth SI, Schumak B, Limmer A, Knolle PA. Liver sinusoidal endothelial cells veto CD8 T cell activation by antigen-presenting dendritic cells. Eur J Immunol. 2008;38:957–67.
Schrage A, Wechsung K, Neumann K, Schumann M, Schulzke JD, Engelhardt B, Zeitz M, Hamann A, Klugewitz K. Enhanced T cell transmigration across the murine liver sinusoidal endothelium is mediated by transcytosis and surface presentation of chemokines. Hepatology. 2008;48:1262–72.
Schurich A, Berg M, Stabenow D, Bottcher J, Kern M, Schild HJ, Kurts C, Schuette V, Burgdorf S, Diehl L, et al. Dynamic regulation of CD8 T cell tolerance induction by liver sinusoidal endothelial cells. J Immunol. 2010;184:4107–14.
Schurich A, Bottcher JP, Burgdorf S, Penzler P, Hegenbarth S, Kern M, Dolf A, Endl E, Schultze J, Wiertz E, et al. Distinct kinetics and dynamics of cross-presentation in liver sinusoidal endothelial cells compared to dendritic cells. Hepatology. 2009;50:909–19.
Semmling V, Lukacs-Kornek V, Thaiss CA, Quast T, Hochheiser K, Panzer U, Rossjohn J, Perlmutter P, Cao J, Godfrey DI, et al. Alternative cross-priming through CCL17-CCR4-mediated attraction of CTLs toward NKT cell-licensed DCs. Nat Immunol. 2010;11:313–20.
Smedsrod B. Clearance function of scavenger endothelial cells. Comp Hepatol. 2004;3 Suppl 1:S22–32.
Tang L, Yang J, Liu W, Tang X, Chen J, Zhao D, Wang M, Xu F, Lu Y, Liu B, et al. Liver sinusoidal endothelial cell lectin, LSECtin, negatively regulates hepatic T-cell immune response. Gastroenterology. 2009;137(1498–1508):e1491–5.
Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol. 2010;10:753–66.
Tokita D, Shishida M, Ohdan H, Onoe T, Hara H, Tanaka Y, Ishiyama K, Mitsuta H, Ide K, Arihiro K, et al. Liver sinusoidal endothelial cells that endocytose allogeneic cells suppress T cells with indirect allospecificity. J Immunol. 2006;177:3615–24.
van Oosten M, van Amersfoort ES, van Berkel TJ, Kuiper J. Scavenger receptor-like receptors for the binding of lipopolysaccharide and lipoteichoic acid to liver endothelial and Kupffer cells. J Endotoxin Res. 2001;7:381–4.
Wang L, Wang X, Xie G, Wang L, Hill CK, DeLeve LD. Liver sinusoidal endothelial cell progenitor cells promote liver regeneration in rats. J Clin Invest. 2012;122:1567–73.
Warren A, Le Couteur DG, Fraser R, Bowen DG, McCaughan GW, Bertolino P. T lymphocytes interact with hepatocytes through fenestrations in murine liver sinusoidal endothelial cells. Hepatology. 2006;44:1182–90.
Wohlleber D, Kashkar H, Gartner K, Frings MK, Odenthal M, Hegenbarth S, Borner C, Arnold B, Hammerling G, Nieswandt B, et al. TNF-induced target cell killing by CTL activated through cross-presentation. Cell reports. 2012;2(3):478–87. doi:10.1016/j.celrep.2012.08.001. Epub 2012 Aug 30.
Wong J, Johnston B, Lee SS, Bullard DC, Smith CW, Beaudet AL, Kubes P. A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. J Clin Invest. 1997;99:2782–90.
Wu J, Meng Z, Jiang M, Zhang E, Trippler M, Broering R, Bucchi A, Krux F, Dittmer U, Yang D, et al. Toll-like receptor-induced innate immune responses in non-parenchymal liver cells are cell type-specific. Immunology. 2010;129:363–74.

Liver Transplantation for Chronic Viral Hepatitis

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Synonyms

Antiviral therapy; Hepatitis B; Hepatitis C; Hepatitis E; Liver transplant

Definition

The essay outlines the indications and outcomes of liver transplantation for hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. Prevention strategies for HBV infection of the liver allograft are successful but not for HCV infection where recurrence is universal. Approaches to antiviral therapy for subsequent HCV infection in the allograft are discussed.

Background

Over 350 million people worldwide are chronically infected with hepatitis B and 200 million

with HCV (Maddrey 2000; El-Serag et al. 2003; Lavanchy 2009). Approximately 20 % will develop the complications of cirrhosis or hepatocellular cancer. Hepatocellular cancer (HCC) is the 3rd most common solid cancer worldwide. Both decompensated cirrhosis and HCC due to HBV and/or HCV are well-recognized indications for liver transplantation although it is clear that liver transplantation, due to its limited availability at a cost/technical level as well as at a donor level, is hardly the solution at a population level to these disease burdens. However, when applied to individuals, the outcomes for HBV now are excellent and HBV recurrence post-transplantation can be reduced to very low levels. By contrast outcomes for HCV-related disease remain suboptimal due to universal HCV recurrence.

Indications of Liver Transplantation

Indications for liver transplantation for chronic hepatitis B and hepatitis C are the presence of hepatic decompensation in a patient with cirrhosis or the development of HCC with or without decompensation (Mazzaferro et al. 1996; Wiesner 2003; Merion et al. 2005). The indications for liver transplantation in the setting of HCC include criteria defined by tumor size and number. The most universal accepted criteria are the Milano Criteria (one tumor $< = 5$ cm in diameter or 3 tumors with maximum diameter of 3 cm.

It is generally thought that patients should be listed for liver transplantation for chronic hepatitis B or chronic hepatitis C cirrhosis once there are single clinical features such as uncontrolled ascites, recurrent/persistent encephalopathy, or persistent gastrointestinal bleeding despite therapeutic interventions. Recently, the Model for End Stage Liver Disease (MELD) scoring system has become a much more evidence-based means of listing patients for transplantation. The MELD is a logarithmic derived number based on the levels of serum bilirubin, INR, and creatinine. The benefit of liver transplantation tends to come in with a MELD score of around 15–17.

Antiviral Therapy for HBV Pre-transplant

Drugs that inhibit HBV DNA polymerase activity by binding to its active site form the basis of antiviral therapy in this setting (Zoulim et al. 2008; Woo et al. 2010). The largest treatment experience in patients with advanced liver disease heading toward liver transplant is with lamivudine. This drug is well tolerated in patients with decompensated cirrhosis and results in undetectable viral DNA in approximately 70 % of patients within 2–3 months. Many studies indicate that such therapy improves symptomatology and blood test results. Furthermore there is a decrease in the need for hospital admissions for complications such as ascites, spontaneous bacterial peritonitis, and encephalopathy. Lamivudine can either stabilize patients on the waiting list, allowing them to proceed to transplantation with detectable HBV DNA and thus minimal chance of disease recurrence, or in many cases avoid transplantation altogether. However, there were certain patients who progress despite antiviral efficacy.

Lamivudine however is limited by the emergence of viral resistance due to one or more mutations in the polymerase gene. These are detected in approximately 20–30 % of patients after 1 year of therapy and in up to 70 % of patients with 5 years. The onset of lamivudine resistance in patients with cirrhosis leads to worsening liver disease and can result in flares that are associated with mortality. There is also a cumulative experience of treating patients' pre-liver transplantation with adefovir. In the setting of hepatic decompensation, it is mainly used as an add-on therapy in patients who develop lamivudine resistance. It is not recommended that such patients switch from lamivudine to adefovir as this is associated with adefovir resistance (30 % at 3 years). The combination of lamivudine and adefovir however is associated with minimal adefovir resistance and may control active viral replication even in decompensated patients.

Two newer agents, entecavir and tenofovir, have very potent HBV antiviral activity. They control HBV viral replication in the vast majority of patients with cirrhosis, and both are associated

with little drug resistance in previously untreated patients. There is now evidence that both these drugs may reverse hepatic decompensation in a similar fashion to lamivudine. Entecavir should not be used in patients with previous lamivudine exposure or resistance as resistance to entecavir has been observed in such patients (50 % at 3 years). Thus, patients with lamivudine resistance are best treated with tenofovir. By 2012, entecavir and tenofovir have become the antiviral agents of choice to reverse hepatic failure and to control HBV replication leading up to liver transplantation.

Natural History of HBV Post-transplant without Antiviral Therapy

Before the use of HBV antiviral therapies, liver transplantation was associated with universal HBV recurrence and a significant rate of severe disease including the description of an unusual pattern of recurrence termed fibrosing cholestatic hepatitis (FCH). This form of HBV resulted in death and allograft loss within 9 months in most patients. The inability to prevent HBV recurrence led many centers, particularly in the USA, to stop performing liver transplantation for HBV disease by the early to mid-1990s.

Antiviral Therapy for HBV Post-liver Transplant

There has been an evolution of approaches to the problem of HBV recurrence over the past 20 years (Angus and Patterson 2008). The first regime to suggest that HBV recurrence could be prevented was the administration of high-dose intravenous Hepatitis B Immune Globulin (HBIG). This was particularly effective in HBV DNA negative and e-antigen negative patients. Thus, by the mid-1990s, high-dose HBIG IVI was the "gold standard" for preventing HBV recurrence and was introduced in many centers. However, it was expensive (up to US$ 50 K per annum) and highly inconvenient requiring repetitive dosing to keep anti-HBsAg titers at >100 U/L and sometimes >250 U/L.

Following the introduction of lamivudine, several studies investigated the use of lamivudine monotherapy in the absence of HBIG to prevent viral replication post-transplantation. This regime was moderately successful but reinfection occurred in up to 50 % of patients. Breakthroughs of HBV with lamivudine-resistant mutations were common, and there was significant mortality when this occurred. One of the exceptions to this was the use of lamivudine monoprophylaxis in Asian recipients who received live donors from hepatitis B surface antigen negative but anti-HBs positive donors.

Lamivudine monotherapy was rapidly replaced by a combination of HBIG and lamivudine. Use of intravenous HBIG at very high doses in combination with lamivudine monotherapy showed almost totally eradication of hepatitis B recurrence and rapidly became the standard of care in most liver transplant centers. More recently, similar efficacy has been shown in several studies involving low-dose HBIG protocols in combination with lamivudine monotherapy or lamivudine in combination with adefovir (for lamivudine resistance). The use of high dose HBIG is now considered obsolete by many centers.

Current issues related to HBV recurrence center around withdrawing or eliminating HBIG. It seems that long-term use of HBIG in the setting is unnecessary. One approach to HBIG withdrawal has been to try and vaccinate patients at some stage post-liver transplantation. This does not usually work unless novel adjuvant vaccines are used. The emergence of more potent HBV antivirals such as entecavir or tenofovir has lead to avoidance of HBIG altogether.

Outcomes of Liver Transplantation: HBV

Liver transplantation for hepatitis B has been one of the most successful changing paradigms in liver transplantation medicine and surgery over the last 20 years (http://www.srtr.org/; http://www.eltr.org; http://www.anzltr.org). Before the introduction of direct antiviral agents, liver transplantation was often contraindicated in

many western countries and outcomes were poor. However, now, outcomes for liver transplantation for hepatitis B are probably one of the most successful in all patient subgroups with 5-year survival rates of over 80 %.

Hepatitis Delta Virus (HDV)

Patients with HBV infection may also be co-infected with HDV (Pascarella and Negro 2010). In the pre-transplant setting, therapies against HDV are not useful. In the post-transplant setting, the recurrence of HDV disease does not occur providing there is no HBV recurrence. HBV recurrence is minimal in this setting even in the absence of preventive approaches outlined previously. However optimal prevention of HBV recurrence is required and should be used. The reason for this is that HDV itself may reinfect the allograft but will only become active if HBsAg returns as the HBsAg is required for the production of HDV replicating virions. If HBV reinfection does occur, however, there can be fulminant HDV disease. This is now uncommon and patients with HDV probably have the best outcomes of all HBV infected patients post-transplant.

Antiviral Therapy for HCV Pre-transplantation

Current antivirals for HCV (interferon-ribavirin based) usually exclude patients with cirrhosis and portal hypertension and certainly patients with hepatic decompensation because of side effects and poor efficacy (Terrault 2008). However, there are a number of patients who proceed to liver transplantation with portal hypertension and HCC that may be suitable for antiviral therapy and are candidates for viral eradication in the pre-transplant period. Several studies with small numbers of patients have been modestly successful in viral eradication in such settings. This particularly applies to non-genotype 1 patients where sustained virologic responses can be achieved on the intention to treat basis in up to 20–30 % of patients. However, in genotype 1 patients, this is more likely to be in the 10–15 % range. If sustained virologic responses are obtained in the pre-transplant setting, it is associated with the prevention of HCV recurrence in the post-transplant setting. Successful therapies in this situation have used the so-called Low-Dose Accelerated Regime ("LADR") starting at half doses of pegylated interferon and half doses of ribavirin and increasing over the first 2–4 weeks depending on tolerability re symptoms and cytopenias.

Natural History of HCV Recurrence Post-liver Transplantation without Antiviral Therapy

HCV reinfection of the allograft can occur within 24 h after liver transplantation resulting in levels of viremia similar to pre-transplant levels within days (Ramirez et al. 2008; Gane 2008; McCaughan et al. 2009). Viral recurrence is then associated with peak viremia at about 3 months which is usually at least one log higher than the pre-transplant setting. This is associated with acute hepatitis. A usual variant at this stage is the development of cholestatic HCV in approximately <10 % of patients which results in high levels of allograft failure.

Studies of the immune-viral response to HCV suggest that early events within the first 3 months post-transplant have a significant effect on the outcome. At the 3–4 month mark increased levels of hepatic inflammation levels of viral replication, lack of a specific HCV immune response, and detection of fibrosis markers are associated with increased levels of allograft loss or fibrosis at later time points. Once chronic hepatitis C is present in the allograft, then there may be progression to cirrhosis over a 5–10-year period in up to 30 % of patients.

Levels of immunosuppression may also effect progression of allograft injury. Pulse corticosteroids and OKT3 therapy are detrimental. However there seems no difference in the type of calcineurin inhibitor used. A recent study suggests that inhibitors of the mammalian target of rapamycin (mTOR) may be beneficial. Polymorphisms within the tumor necrosis

factor (TNF) gene have also been associated with increased inflammation in the liver allograft. The occurrence of CMV infection is also a predictor factor for worse outcomes.

Antiviral Therapy Post-liver Transplant

Early use of interferon-based therapies in attempt to minimize viral replication during the early months post-transplant seems not to be beneficial probably related to poor tolerability (Terrault 2008). The use of antiviral therapy in the post-transplant once there is established chronic infection and inflammation in the allograft is considerably lower than in the non-transplant setting. Sustained virologic response rates are only obtained in 15–20 % of genotype 1 patients and 30–40 % of genotype 2/3 patients. However, if a sustained virologic response is obtained then long-term outcomes in such patients are much improved compared to untreated patients or treated patients without a sustained virologic response. Polymorphisms in the IL28 gene locus of both donor and recipient are associated with differing responses to interferon in the post-transplant setting.

Outcomes from Liver Transplantation: Hepatitis C

Unlike hepatitis B infection outcomes for liver transplant for hepatitis C are worse than non-hepatitis C patients over long periods of times (http://www.srtr.org/; http://www.eltr.org; http://www.anzltr.org; McCaughan et al. 2010). Patients with hepatitis C have 5-year survival rates approximately 70 % compared to 80 % for non-hepatitis C patients and at the 10-year mark 50 % versus 70 %.

HCV Prevention of Recurrence: The Future

Direct antivirals against HCV without interferon are on the horizon within 5 years. Proof of concept studies already exist. The successful introduction of such therapies will allow the elimination of HCV and recurrence post-transplantation. Whether such agents can prevent disease progression at an advanced stage pre-transplant and avoid transplantation in a similar fashion to HBV remains to be seen.

Hepatitis E Virus (HEV) and Liver Transplantation

HEV has been associated with chronic hepatitis in the hepatic allograft (Haagsma et al. 2008).

Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Animal models of Hepatitis B and C
- ▶ Immune Responses to the Hepatitis C Virus
- ▶ Immunosuppression in Clinical Liver Transplantation
- ▶ Liver Transplantation Tolerance in Animal Models for Encyclopedia of Medical Immunology
- ▶ Mammalian Target of Rapamycin (mTOR)

References

Angus PW, Patterson SJ. Liver transplantation for hepatitis B: what is the best hepatitis B immune globulin/antiviral regimen? Liver Transpl. 2008;14(Suppl 2):S15–22.

El-Serag HB, Davila JA, Petersen NJ, et al. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med. 2003;139(10):817–23.

Gane EJ. The natural history of recurrent hepatitis C and what influences this. Liver Transpl. 2008;14(Suppl 2):S36–44.

Haagsma EB, van den Berg AP, Porte RJ, et al. Chronic hepatitis E virus infection in liver transplant recipients. Liver Transpl. 2008;14:547–53.

Lavanchy D. The global burden of hepatitis C. Liver Int. 2009;29(Suppl 1):74–81.

Maddrey WC. Hepatitis B: an important public health issue. J Med Virol. 2000;61(3):362–6.

Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular

carcinomas in patients with cirrhosis. N Engl J Med. 1996;334:693–9.
McCaughan GW, Shackel NA, Bertolino P, Bowen DG. Molecular and cellular aspects of hepatitis C virus reinfection after liver transplantation: how the early phase impacts on outcomes. Transplantation. 2009;87:1105–11.
McCaughan GW, Shackel NA, Strasser SI, et al. Minimal but significant improvement in survival for non-hepatitis C-related adult liver transplant patients beyond the one-year posttransplant mark. Liver Transpl. 2010;16:130–7.
Merion RM, Schaubel DE, Dykstra DM, et al. The survival benefit of liver transplantation. Am J Transplant. 2005;5:307–13.
Pascarella S, Negro F. Hepatitis D virus: an update. Liver Int. 2010;2010(29):1478–3231.
Ramirez S, Perez-Del-Pulgar S, Forns X. Virology and pathogenesis of hepatitis C virus recurrence. Liver Transpl. 2008;14(Suppl 2):S27–35.
Terrault NA. Hepatitis C, therapy before and after liver transplantation. Liver Transpl. 2008;14(Suppl 2): S58–66.
Wiesner R. Model for end-stage liver disease (MELD) and allocation of donor livers. Gastroenterology. 2003;124(1):91–6.
Woo G, Tomlinson G, Nishikawa Y, et al. Tenofovir and entecavir are the most effective antiviral agents for chronic hepatitis B: a systematic review and Bayesian meta-analyses. Gastroenterology. 2010;139(4):1218–29.
Zoulim F, Radenne S, Ducerf C. Management of patients with decompensated hepatitis B virus associated [corrected] cirrhosis. Liver Transpl. 2008;2(2): S1–7.

Liver Transplantation Tolerance in Animal Models for Encyclopedia of Medical Immunology

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Definition

This is a short review of the tolerance that can follow experimental liver grafting and the "operational tolerance" that can occur after clinical liver grafting. Although there are many data on the subject that are considered, a full explanation of the mechanisms involved has not yet been clarified.

Liver transplantation in the laboratory and clinic has provided an immunological puzzlement for all those involved, not least the patients. Initially in the early 1960s, the technical details of the surgery required prolonged and consistent perseverance in an understanding of the requirements of the liver being transplanted and the maintenance of a viable physiology of the recipients subjected to the operation. Dr. Starzl in Denver and Dr. Moore in Boston independently worked out successful techniques for transplantation of the liver in the dog. In both cases it was necessary to bypass blood from the lower part of the body and the bowel to the superior vena cava during the anhepatic phase after the recipient's liver had been removed and before the donor liver was revascularized. The standard immunosuppression then available of azathioprine prolonged graft survival modestly in experimental animals successfully transplanted and in one celebrated case of Dr. Starzl, a dog lived for 13 years after immunosuppression was stopped. The technique was then developed in the pig (Cordier et al. 1966) in Paris, in Bristol (Peacock and Terblanche 1967), and in Cambridge (Calne et al. 1967); although the surgery was difficult, success was achieved. In Cambridge Binns (1973) had been studying immunological tolerance in the pig using the classical Medawar intrauterine fetal injection. This was extended to kidney and liver transplants. Although it was possible to demonstrate tolerance with kidney allografts more easily than with skin, liver transplants in the pig sometimes survived long periods without the recipient receiving any immunosuppressive drugs at any stage. In most cases the animals developed rejection confirmed histologically and biochemically, but this subsided spontaneously (Calne et al. 1969).

Although pigs have been bred over hundreds of years to improve the quality of the meat, they are in no sense inbred, as are laboratory murine strains. The porcine liver graft could also protect other tissues from the same donor from rejection,

skin grafts lasted longer but were rejected, while the liver continued to survive. Kidney grafts also had prolonged survival when transplanted together with the liver. These observations were supplemented by many studies in rats where inbred strains were available, and it was shown that between certain strains, irreversible rejection occurred, and between others there was little evidence of rejection. Some rat liver transplants behaved in a similar manner to the pig with rejection and then spontaneous recovery. Reports of these experiments provoked the Lancet to write a leading article entitled "Strange English Pigs" (Anon 1969); however, the phenomenon was not limited to the nationality of the pigs and was repeated in other laboratories (Houssin et al. 1980; Zimmerman et al. 1981; Kamada and Wright 1984; Kamada et al. 1981). An important, detailed, and comprehensive review was published in 2007 (Benseler et al. 2007).

Operational Tolerance in Clinical Liver Grafting

The burden of immunosuppression, apart from its high expense, is a severe impediment to the quality of life of transplant patients. The usual daily dose of pills is unwelcome and unpleasant, not only requiring the swallowing of numerous medications but also the knowledge that each separate drug has side effects, all of which are unpleasant and some extremely unpleasant, for example, the cushingoid facies, stunting of growth and spontaneous fractures in patients taking large doses of steroids, hirsutism, and gum hypertrophy of patients receiving cyclosporine. Both the commonly used calcineurin-inhibiting drugs, cyclosporine and tacrolimus, are nephrotoxic even in therapeutic doses, and they can cause diabetes; azathioprine can inhibit the bone marrow with severe pancytopenia; mycophenolate mofetil can cause gastrointestinal disturbances. Patients know of all these side effects and also the fact that immunosuppression in general renders them susceptible to infection and a variety of malignancies, particularly lymphoproliferative disorders.

It is not surprising then that noncompliance is common in recipients of all organ transplants, but it is a particular cause of graft failure in teenagers and especially in girls. A number of patients transplanted in the Denver/Pittsburgh series of liver transplants stopped taking their drugs after a variety of time intervals from the operation. Some of them rejected their grafts, but others did not suffer from rejection even after many years. These patients were "operationally tolerant" following liver grafting. Other cases in the same series had their immunosuppression stopped by the medical team because of severe viral infections or malignancy. An analysis of this liver transplant-induced "operational tolerance" was undertaken, and a deliberate attempt was made to wean patients who had prolonged survival and good liver function with minimal immunosuppression. Some of these patients did well, exhibiting operational tolerance, but others developed rejection and needed to be returned to immunosuppressive treatment (Mazariegos et al. 1997).

Studies on cell trafficking between the liver and the recipient were published by the Denver/Pittsburgh group whose hypothesis on microchimerism in the blood produced by these cells has been controversial in terms of unravelling cause and effect (Starzl et al. 1993). Undoubtedly the phenomenon of micro-chimaerism occurs but whether it is the cause of specific immunosuppression or an epiphenomenon is still disputed. The protective effect in patients with liver transplantation on grafts or other organs from the same donor was also documented (Rasmussen et al. 1995). Strangely the outcome of liver grafts, unlike that of other organs, does not seem to be beneficially influenced by HLA-matching between donor and recipient.

Liver transplants release large amounts of soluble MHC Class 1 molecules which can have immunosuppressive properties (Zavazava et al. 1996; Geissler et al. 1997). The argument in favor of the immunosuppressive effect of micro-chimerism was discussed by Starzl and colleagues in the Lancet in 1992 (Starzl et al. 1992).

The relevance of micro-chimerism to the fate of organ transplantation and especially liver transplants has been much debated since Starzl's theory was first published. Micro-chimerism can certainly be demonstrated after liver and other solid organ transplants, but there does not seem to be a clear-cut relevance of the presence or absence of micro-chimerism and the immunological fate of the graft (Schlitt et al. 1994). The role of regulatory T cells has also been studied, and experimentally, these Treg cells have been implicated in the acceptance of rat liver grafts (Gassel et al. 1992).

A study by Sriwatanawongsa et al. (1995) compared the tolerogenic potential of leukocytes from the liver compared with liver parenchymal cells in rats. Parenchymal cells were able to induce tolerance, while passenger lymphocytes did not prolong donor skin grafts. Similar findings were reported from other laboratories.

Conclusions

There seems to be a strong body of agreement relating to spontaneous antigen-specific liver transplant tolerance. It has been demonstrated independently in different laboratories in a variety of species, particularly the pig, rat, and mouse, and also "operational tolerance" has been reported in clinical liver transplantation. Some of these observations and suggested explanations have been reviewed in this text, but it must be admitted that although the phenomenon is important practically and theoretically, the mechanisms involved are not fully understood. There seems to be a delicate balance between acceptance and rejection of a liver graft and also evidence of an active immunological engagement between donor and recipient that is relevant to the development of liver-induced tolerance. In the 52 years since the phenomenon was first described experimentally, there is still not a complete picture; however, when robust tolerance occurs, it is an important and striking biological phenomenon that would warrant further study, since harnessing of the mechanisms, if they were understood, might be utilized to induce tolerance, not only to the liver but to other organ and tissue transplants.

Cross-References

▶ Immunosuppression in Clinical Liver Transplantation

References

Anon. Strange english pigs. Lancet. 1969;2:940–1.
Benseler V, McCaughan GW, Schlitt HJ, et al. The liver: a special case in transplantation tolerance. Semin Liver Dis. 2007;27(2):194–213.
Binns RM. Cellular immunology in the pig. Proc R Soc Med. 1973;66(12):1156–60.
Calne RY, White HJO, Yoffa DE, et al. Observations of orthotopic liver transplantation in the pig. Br Med J. 1967;2:478–80.
Calne RY, Sells RA, Pena JR, et al. Induction of immunological tolerance by porcine liver allografts. Nature. 1969;223:472–4.
Cordier G, Garnier H, Clot JP, et al. La greffe de foie orthotopique chez le porc. Mem Acad Chir (Paris). 1966;92:799–807.
Gassel HJ, Hutchinson IV, Engemann R, Morris PJ. The role of T suppressor cells in the maintenance of spontaneously accepted orthotopic rat liver allografts. Transplantation. 1992;54:1048–53.
Geissler EK, Korzun WJ, Graeb C. Secreted donor-MHC class I antigen prolongs liver allograft survival and inhibits recipient anti-donor cytotoxic T lymphocyte responses. Transplantation. 1997;64:782–6.
Houssin D, Gigou M, Franco D, et al. Specific transplantation tolerance induced by spontaneously tolerated liver allograft in inbred strains of rats. Transplantation. 1980;29:418–9.
Kamada N, Wright DG. Antigen-specific immunosuppression induced by liver transplantation in the rat. Transplantation. 1984;38:217–21.
Kamada N, Davies HS, Roser B. Reversal of transplantation immunity by liver grafting. Nature. 1981;292:840–2.
Mazariegos GC, Reyes J, Marino IR, et al. Weaning of immunosuppression in liver transplant recipients. Transplantation. 1997;63:243–9.
Peacock JH, Terblanche J. Orthotopic homotransplantation of the liver in the pig. In: Read AE, editor. The liver. London: Butterworth; 1967. p. 333.
Rasmussen A, Davies HF, Jamieson NV, et al. Combined transplantation of liver and kidney from the same donor protects the kidney from rejection and improves kidney graft survival. Transplantation. 1995;59:919–21.

Schlitt HJ, Hundrieser J, Ringe B, et al. Donor-type microchimerism associated with graft rejection eight years after liver transplantation. N Engl J Med. 1994;330:646–7.
Sriwatanawongsa V, Davies HS, Calne RY. The essential roles of parenchymal tissue sand passenger leukocytes in the tolerance induced by liver grafting in rats. Nat Med. 1995;1:428–32.
Starzl TE, Demetris AJ, Murase N, et al. Cell migration, chimerism, and graft acceptance. Lancet. 1992;339:1579–82.
Starzl TE, Demetris AJ, Trucco M, et al. Cell migration and chimerism alter whole-organ transplantation: the basis of graft acceptance. Hepatology. 1993;17: 1127–52.
Zavazava N, Kronke M, Soluble HLA. Class I, molecules induce apoptosis in alloreactive cytotoxic T lymphocytes. Nat Med. 1996;2:1005–10.
Zimmerman FA, Davies HS, Knoll PP, et al. Orthotopic liver allografts in the rat: the influence of strain combination on the fate of the graft. Transplantation. 1984;38:217–21.
This is a very full and well-written review.

Liver Vasculature and Microvasculature

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Synonyms

Central vein; Central venule; Fat-storing cell; Hepatic; Ito cell; Liver; Stellate cell; Terminal hepatic vein

Definition

The hepatic vasculature comprises all of the blood vessels that supply blood to and from the liver plus lymphatics which drain lymph from the organ. Within the liver, blood vessels smaller than 300 μm in diameter are classified as the microvasculature. This entry provides an overview of the dynamic structure, function, and regulation of the hepatic vasculature, particularly its microvasculature, in health and some of the basic responses to disease.

Blood Supply to the Liver

The liver has a dual blood supply. Approximately 80 % of the blood entering the liver is poorly oxygenated blood supplied by the portal vein containing venous blood flowing from the intestines, pancreas, spleen, and gall bladder. The remaining 20 % of the blood supply is well oxygenated and delivered by the hepatic artery. Both the hepatic portal vein and hepatic artery enter the liver at the hilus, where efferent bile ducts as well as lymphatics also exit the organ. These vessels then branch to supply the lobes of the liver.

Within each lobe, branches of the hepatic artery, hepatic portal vein, bile duct, and lymphatic vessels travel together in portal tracts through the liver parenchyma. After repeated branching to form the microvasculature, terminal branches of the blood vessels (portal venules and hepatic arterioles) supply blood to the sinusoids which are the exchange vessels in the liver and are organized in an extensive anastomotic network which nourishes the plates of parenchymal cell on several sides. Branches of hepatic arterioles also supply the peribiliary plexus of capillaries nourishing the bile ducts and then drain into sinusoids or occasionally into portal venules. After flowing through the sinusoids, blood is collected in small branches of hepatic veins termed central venules (central veins, terminal hepatic venules). These course independently of the portal tracts and drain via hepatic veins, which leave the liver on the dorsal surface and join the inferior vena cava. Occasionally, branches of hepatic arterioles bypass the sinusoidal bed to supply the walls of hepatic veins.

Lymphatic vessels originate as blind-ending capillaries in the connective tissue spaces of portal tracts. The fluid contained in these lymphatics flows toward the hepatic hilus and eventually into the cisternae chyli.

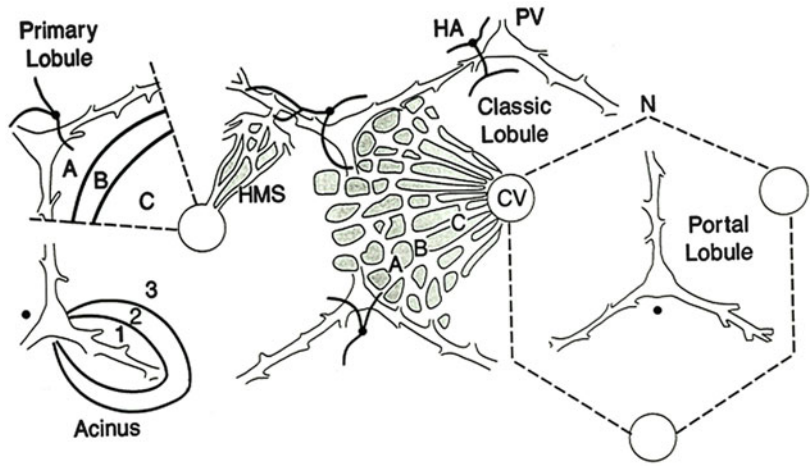
Further details and references concerning the blood supply to the liver are reviewed in McCuskey (2012).

Microvascular Functional Units

The organization of each liver lobe into structural or functional units related to function and/or disease has been the subject of considerable debate during the past century. Several models, none of which are mutually exclusive, have been proposed as follows and illustrated in Fig. 1.

The classic hepatic lobule is a polygonal structure having as its central axis a central venule, with portal tracts distributed along its peripheral boundary. The peripheral boundaries of these lobules are poorly defined in most species, including man. In some species, e.g., the pig, there is considerably more connective tissue present in the liver, and the connective tissue is distributed along the peripheral boundary of classic lobules thus making them very distinct. Considerable sinusoidal anastomoses occur between adjacent lobules, and thus the blood collected by each central venule is supplied by several portal venules. For these reasons, and because of intralobular regional differences in oxygenation, metabolic functions, and responses to some disease, an acinar concept was proposed to define the hepatic functional unit.

The hepatic acinus is a unit having no distinct morphologic boundaries. Its axis is a portal tract, and its peripheral boundary is circumscribed by an imaginary line connecting the neighboring terminal hepatic venules (central hepatic venules of the classic lobule), which collect blood from sinusoids. Contained within the acinus are three zones, each having different levels of oxygenation and metabolic function. Although the acinar concept has been widely accepted, it fails to account for those mammalian species (e.g., pig, seal) that have connective tissue boundaries circumscribing the classic lobule.



Liver Vasculature and Microvasculature, Fig. 1 Contiguous hepatic lobules illustrating the interconnecting network of sinusoids derived from two portal venules (PV). Note that the sinusoids become more parallel as they course toward the central venule (CV), which forms the axis of the classic lobule. Hepatic arterioles (HA) supply blood to sinusoids near the periphery of the lobule, usually by terminating in inlet venules or terminal portal venules. As a result, three zones (*1*, *2*, *3*) of differing oxygenation and metabolism have been postulated to compose a hepatic acinus, with its axis being the portal tract (*lower left*). Several acini would compose the portal lobule (*lower right*). Each classic lobule contains several cone-shaped subunits having convex surfaces fed by portal and arterial blood at the periphery and its apex at the central venule (*upper left*). *A*, *B*, and *C* represent hemodynamically equipotential lines in a "primary lobule." Recently, a modification further subdivides lobules into conical hepatic microcirculatory subunits (HMS), each being supplied by a single inlet venule (McCuskey 2008)

Additional inconsistencies increasingly have been identified in three-dimensional studies of metabolic heterogeneity and microvascular structure.

In yet another model of hepatic organization, the unit is defined by bile drainage. The so-called portal lobules have at their center a portal tract, with central veins present around the periphery of each lobule. This concept has received little support.

Currently, the concept of subunits of the classic lobule forming functional units is the most consistent with existing evidence. In this model, each "classic" lobule consists of several "primary lobules." Each primary lobule is cone shaped, having its convex surface at the periphery of the classic lobule supplied by terminal branches of portal venules and hepatic arterioles and its apex at the center of the classic lobule drained by a central (terminal hepatic) venule. These "primary lobules" were renamed as "hepatic microvascular subunits (HMS)" and were demonstrated to consist of a group of sinusoids supplied by a single inlet venule and its associated termination of a branch of the hepatic arteriole from the adjacent portal space. Further confirmation of this HMS concept was obtained studying their development in neonatal livers. Accompanying the HMS are hepatic parenchymal cells and the associated cholangioles and canaliculi. Hepatocellular metabolic gradients also have been demonstrated to conform to this proposed functional-unit concept.

Studies using three-dimensional reconstruction of sectioned livers, scanning electron microscopic examination of corrosion casts, and in vivo microscopy of several species support the concept of the functional unit being a conical microvascular subunit of the classic lobule. These "primary lobules" were renamed "hepatic microvascular subunits (HMS)" and were demonstrated to consist of a group of sinusoids supplied by a single inlet venule and its associated termination of a branch of the hepatic arteriole (AST) from the adjacent portal space.

Further details and references concerning microvascular functional units are reviewed in Ekataksin et al. (1997) and McCuskey (2012).

Hepatic Microvascular System

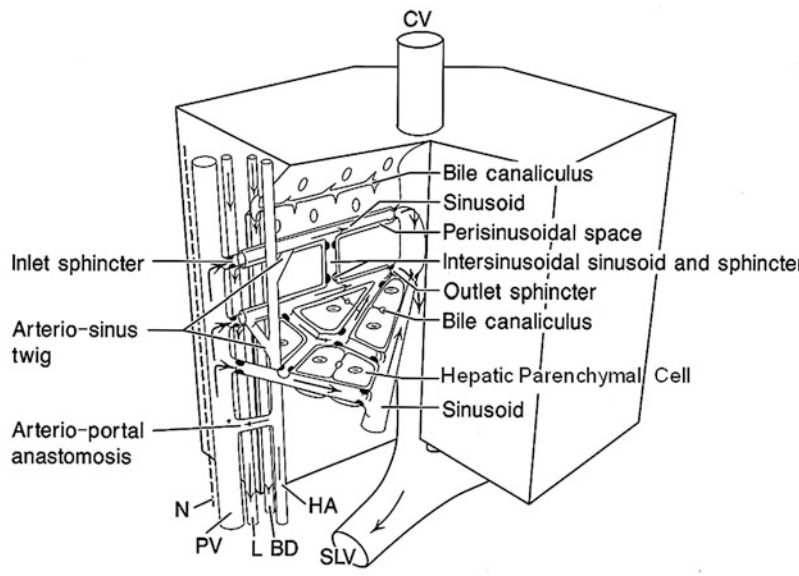
The hepatic microvascular system comprises all blood and lymphatic vessels immediately involved in the delivery and removal of fluids to and from the hepatic parenchyma, namely, portal venules, hepatic arterioles, sinusoids, central venules, and lymphatics. Figure 2 is a diagram illustrating the microvascular connections that supply and drain the sinusoids within a single hepatic lobule.

Most blood enters the sinusoids from portal venules. These inlets are reported to be guarded by sphincters composed of sinusoidal lining cells termed the afferent or inlet sphincters. Arterial blood enters some of the sinusoids, principally through branches of the hepatic arterioles. These vessels, arterio-sinus twigs, terminate in sinusoids near their origins from portal venules. In addition, occasional direct connections (arterioportal anastomoses, APA) have been observed with the terminal portal venules. The frequency of these APAs appears to be species dependent. Because all of these structures are independently contractile, the sinusoids receive a varying mixture of portal venous and hepatic arterial blood. Finally, some evidence suggests that the fraction of blood delivered to the sinusoids by the hepatic artery differs between the hilus and periphery of hepatic lobes. Within the network of sinusoids, blood flow is reportedly regulated by contractile sinusoidal lining cells which control not only the velocity of flow but also its distribution within the network. Blood leaves the sinusoids by flowing into central (terminal hepatic) venules reportedly by passing through outlet or efferent sphincters composed of sinusoidal lining cells.

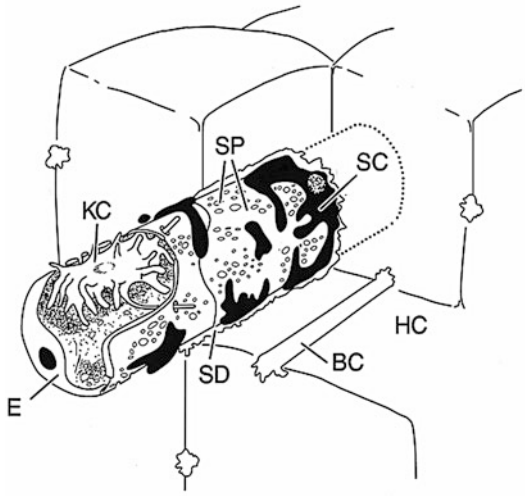
Further details and references concerning the hepatic microvascular system are reviewed in McCuskey (2000, 2008, 2012).

Structure and Function of Hepatic Sinusoids

Structure. The sinusoids are unique exchange vessels composed of specialized non-parenchymal



Liver Vasculature and Microvasculature, Fig. 2 Hepatic microvasculature as determined by in vivo microscopic studies. PV portal venule, HA hepatic arteriole, L lymphatic, BD bile ductule, N nerve, CV central venule, SLV sublobular hepatic vein. *Arrows* indicate direction of flow (McCuskey 2008)



Liver Vasculature and Microvasculature, Fig. 3 Sinusoid and contiguous hepatic parenchymal cells (HC). E endothelium, KC Kupffer cell, SD space of Disse, SP sieve plate composed of endothelial fenestrae, SC stellate cell, BC bile canaliculus (McCuskey 2008)

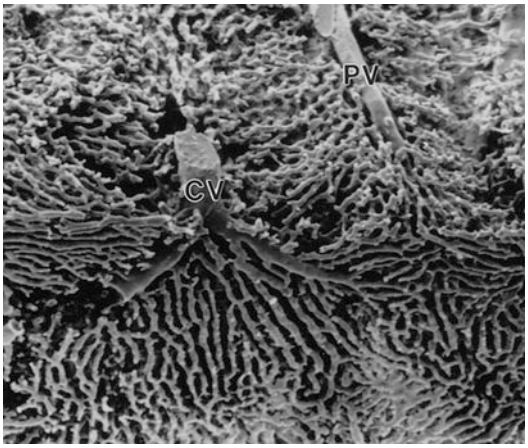
cells that exhibit structural and functional heterogeneity. The structure of the sinusoid is illustrated in Fig. 3.

The liver sinusoidal endothelial cells (LSEC) are highly fenestrated and lack a supporting basal lamina. The fenestrae are organized in clusters known as sieve plates. As a result, there is continuity between the plasma in the sinusoid lumen and the perisinusoidal space (of Disse). The sinusoidal endothelial cells contain numerous endosomes and scavenge a number of substances including breakdown products of connective tissue. They also are the source of several cytokines, eicosanoids, nitric oxide, and endothelins.

Stellate cells (fat-storing cells of Ito) lie external to the endothelium in the space of Disse. They are pericytes which frequently contain lipid droplets that are a storage site for vitamin A. Multiple cytoplasmic projections of these cells surround and embrace the abluminal surfaces of the endothelial cells. When activated, these cells produce collagen and become contractile. As a result, they are thought to play a role in the regulation of sinusoidal blood flow.

Kupffer cells are attached to the luminal surfaces of the endothelium. These are highly phagocytic, specialized fixed macrophages of the liver and contain numerous lysosomes and phagosomes. Kupffer cells are involved in a number of host defense mechanisms and immune functions and are the source of a number of cytokines, eicosanoids, free radicals, and nitric oxide.

Further details and references concerning the structure of hepatic sinusoids are reviewed in Wisse et al. (1996) and McCuskey (2012).



Liver Vasculature and Microvasculature, Fig. 4 Vascular cast of the hepatic microvasculature illustrating the tortuous, anastomotic sinusoids adjacent to the portal venule (PV) and the more parallel and larger sinusoids near the central venule (CV) (McCuskey 2008)

Heterogeneity. The organization of the sinusoid network exhibits heterogeneity. Near portal venules and hepatic arterioles, sinusoids are arranged in interconnecting polygonal networks; farther away from the portal venules, the sinusoids become organized as parallel vessels that terminate in central venules (terminal hepatic venules). Short inter-sinusoidal sinusoids connect adjacent parallel sinusoids. Figure 4 is a microvascular cast illustrating these regional differences.

In the periportal area, the volume of liver occupied by sinusoids is greater than that surrounding central venules. However, because of the smaller size and anastomotic nature of the periportal sinusoids, the surface available for exchange in this area (surface/volume ratio) is greater than in centrilobular sinusoids. The size and pattern of distribution of endothelial fenestrae differs along the length of the sinusoid. At the portal end, the fenestrae are larger but comprise less of the endothelial surface area than they do in the pericentral region. The functional significance of these regional differences is unclear but relates to the functional metabolic heterogeneity that has been demonstrated for hepatocytes in different regions of the lobule. This, in turn, may depend on the recognized portal-to-central intralobular oxygen gradient and the unique microcirculation in the liver.

Further details and references concerning heterogeneity are reviewed in McCuskey (2008) and (2012).

Morphologic sites for regulating the hepatic microcirculation. There are several potential morphological sites for regulating blood flow through the sinusoids. These include the various segments of the afferent portal venules and hepatic arterioles, the sinusoids themselves, as well as central and hepatic venules. These vessels contain several potentially contractile cells – smooth muscle cells in arterioles and venules – and in sinusoids, endothelial, stellate, and Kupffer cells.

Portal venules and central venules contain limited amounts of smooth muscle in their walls relative to their luminal size but nevertheless are contractile and respond to pharmacologic agents. Hepatic arterioles are more responsive because of a complete investment of smooth muscle and relatively small lumens. The principal site of regulation of blood flow through the sinusoids, however, is thought to reside in the sinusoid itself, where the major blood pressure drop occurs in the liver.

The sinusoidal lining cells are responsive to a wide variety of pharmacodynamic substances. By contracting (or swelling), they may selectively reduce the patency of the sinusoid lumen, thereby altering the rate and distribution of blood flow. The relative roles of Kupffer versus endothelial cells in this process are not yet resolved, but both appear to be involved. The participation of perisinusoidal, stellate cells (fat-storing, Ito cells) in regulating sinusoidal diameter also has been reported. All three cell types contain filaments, tubules, and contractile proteins suggestive of contractile activity.

Because of these structures, blood flow through individual sinusoids is variable. At sites where the lumen is narrowed by the bulging, nuclear regions of sinusoidal lining cells, flow may be impeded by leukocytes that transiently plug the vessel and obstruct flow. Transient leukocyte plugging is more frequent in the periportal sinusoids, which are narrower and more tortuous

than those in the centrilobular region. The more plastic erythrocytes usually flow easily through such sites unless the lumen is reduced to near zero. Some sinusoids, however, may act as thoroughfare channels and have relative constant rates of blood flow, while others have more intermittent flow. This may depend on not only the distribution of intra-sinusoidal sphincter cells but also on the distribution of arterio-sinus twigs (AST) and the contribution of arterial blood flowing to individual sinusoids. For example, arterial blood flowing into an individual sinusoid through a dilated AST may increase the rate of sinusoidal blood flow. Because of the delivery of arterial blood at higher pressure, some arterial blood may even reverse the entry of portal blood into the sinusoids. As a result, the AST in concert with the initial segment of the sinusoid in which it terminates may form a "functional" arterioportal anastomosis so that arterial blood is delivered into the portal venules. In the anesthetized, healthy animal, however, terminal branches of the hepatic arteriole containing flow are seen infrequently so that most blood delivered to the sinusoids is derived from the portal venules. Consistent with this is the in vivo microscopic observation that the velocity of flow in sinusoids and portal and central venules located near the capsule of the liver is not significantly altered by hepatic artery occlusion in healthy anesthetized rats. However, arterial inflow to the sinusoids may be more significant in regions near the hepatic hilum.

The frequency distribution of the wide variations in blood flow in the sinusoids exhibits a polymodal pattern composed of several Gaussian distributions. These wide variations in flow are due to the structural features previously described for sinusoids and also are due to intermittent arterial inflow into the sinusoids. Blood pressures in portal and central venules have been measured to be about 6–7 cm H_2O and 1.5–3.0 cm H_2O, respectively. Arterial blood enters the sinusoid at pressures ranging from 12 cm to 25 cm H_2O.

Further details and references concerning regulation of sinusoidal blood flow are reviewed in Clemens and Zhang (1999), Vollmar and Menger (2009), and McCuskey (2000, 2012).

Pathophysiology of the Hepatic Microcirculation

Significant interactive roles for endotoxin, cytokines, chemokines, reactive-free radicals, nitric oxide (NO), endothelin (ET-1), carbon monoxide (CO), sinusoidal lining cells, leukocytes, and platelets have been demonstrated in the pathophysiology of hepatic microvascular disturbances and parenchymal injury resulting from infection, toxicants, and ischemia/reperfusion following hemorrhage or liver transplantation. The responses of the hepatic microvasculature are of two basic types: (a) an inflammatory response involving paracrine activation of the LSEC by mediators released from adjacent Kupffer cells and/or hepatic parenchymal cells following stimulation by toxicants leading to the upregulation of adhesion molecules and the subsequent adhesion of leukocytes to the LSEC as well as swelling of the LSEC both of which restrict sinusoidal blood flow and (b) direct injury of the LSEC resulting in loss of fenestrae, formation of gaps, penetration of the sinusoidal lining by blood cells, destruction of LSEC, and obstruction of the sinusoid by SEC debris. The inflammatory response results from endotoxemia, sepsis, ischemia/reperfusion injury, and acute alcohol ingestion, while direct injury is elicited by acetaminophen (APAP) or during hepatic venoocclusive disease by pyrrolizidine alkaloids. Both types of injury may occur together or sequentially as is seen when ethanol sensitizes sinusoidal lining cells to other toxicants such as APAP or endotoxin which exacerbates the extent of injury. In should be noted that due to the highly anastomotic nature of the hepatic sinusoid bed, plugging of single or scattered segments of sinusoids results in redirection of blood flow into adjacent unplugged vessels. However, unless the injury is highly localized, these latter vessels eventually become plugged and results in microcirculatory failure.

Further details and references concerning pathophysiology of the hepatic microcirculation are reviewed in Clemens and Zhang (1999), Vollmar and Menger (2009), and McCuskey (2008, 2012).

Cross-References

- ▶ Endothelial Cells and Inflammation
- ▶ Hepatic Lymphatic System
- ▶ Kupffer Cells in Immune Tolerance
- ▶ Neutrophils in Endothelial Damage
- ▶ Nitric Oxide
- ▶ Ultrastructure of the Liver Sinusoid

References

Clemens M, Zhang J. Regulation of sinusoidal perfusion: in vivo methodology and control by endothelins. Semin Liver Dis. 1999;19:383–96.

Ekataksin W, Zou Z, Wake K, Chunhabundit P, Somana R, Nishida J, McCuskey R. The hepatic microcirculatory subunits: an over-three-century-long search for the missing link between the exocrine unit and an endocrine unit in mammalian liver lobules. In: Motta P, editor. Recent advances in microscopy of cells, tissues and organs. Rome: Univ Rome "La Sapienza"; 1997. p. 407–12.

McCuskey R. Morphologic mechanisms for regulating blood flow through hepatic sinusoids. Liver. 2000;20:3–7.

McCuskey R. The hepatic microcirculation in health and in response to toxicants. Anat Rec. 2008;291:661–71.

McCuskey R. Anatomy of the liver. In: Boyer T, Mann M, Sanyal A, editors. Zakim and Boyer's hepatology. 6th ed. Philadelphia: Elsevier; 2012. p. 3–19.

Vollmar B, Menger M. The hepatic microcirculation: mechanistic contributions and therapeutic targets in liver injury and repair. Physiol Rev. 2009;89:1269–339.

Wisse E, Braet F, Luo D, DeZanger R, Jans D, Crabbe E, Vermoesen A. Structure and function of sinusoidal lining cells in the liver. Toxicol Pathol. 1996;24:100–11.

Lupus Erythematosus, Skin

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Synonyms

Pathogenesis of cutaneous lupus erythematosus

Definition

Cutaneous lupus erythematosus (CLE) is an inflammatory skin condition in which a characteristic inflammatory infiltrate of primarily lymphocytes damages the interface between the top layer of the skin (epidermis) and the dermis. CLE is important as it may be manifestation of systemic lupus erythematosus, is often an unwanted reaction to ultraviolet light exposure of the skin, and can cause permanent scarring and disfigurement.

Introduction

Although cutaneous lupus erythematosus (CLE) is the second most frequent manifestation of systemic lupus erythematosus (SLE), it may present as an organ-specific (e.g., skin limited) disease. CLE may present with acute, subacute, or chronic lesions that each have distinct clinical and pathological characteristics but are thought to have a similar pathogenesis (Obermoser et al. 2010; Wenzel et al. 2010). The immunepathogenesis of CLE is still not completely understood. However, due to the accessibility of the skin for study, much has been learned about the inflammatory response in CLE. In general, environmental stimulation is thought to initiate an inflammatory response with subsequent recruitment of inflammatory cells into the skin. Two phases of an inflammatory response, initiation of inflammation followed by induction of tissue injury, participate in the formation of skin lesions in CLE. Herein, published information pertinent to each of these proposed phases of lesion induction is reviewed.

Initiation of Inflammation in CLE

Environmental factors trigger an immune response in genetically predisposed individuals. Ultraviolet (UV) light is considered the most common environmental trigger for SLE and CLE. Other factors associated with the onset of CLE include viruses, drugs, trauma, chemicals,

cigarette smoking, psychological stress, and hormones. These factors may induce local alterations in epidermal KCs or resident cells that, in turn, induce immune mediators and cytokines to recruit inflammatory cells.

UV Light as Initiator of CLE Lesions and SLE

The most consistent environmental trigger factor for SLE is UV light, and this has been the most extensively studied environmental initiator of disease. One of the hallmarks of patients with SLE is the development of cutaneous lesions upon exposure to sunlight. Up to 70 % of patients with SLE report photosensitivity, and abnormal photoreactivity is regularly noted in patients with either SLE or CLE (Kuhn et al. 2010). In murine models of lupus, repeated UV exposure can accelerate the spontaneous onset of SLE in lupus mouse models (Ghoreishi and Dutz 2010). How does this occur?

UV-Mediated Induction of Cell Death

Sunlight can induce or exacerbate skin lesions in SLE patients by inducing skin cell (keratinocyte – KC) death through either apoptosis or necrosis. Apoptotic cells are increased in LE skin lesions suggesting that these cells may not be cleared appropriately or may be more susceptible to cell death. Abnormalities in dead cell clearance have been noted in SLE patients (Gaipl et al. 2007) and may predispose to inflammation (Shao and Cohen 2011). UV irradiation of patients with SLE does not reproducibly induce more skin cell death than in unaffected individuals (therefore it is unlikely that skin in SLE is simply more susceptible to cell death), but it does induce an inflammatory environment (Bijl et al. 2007).

In addition to direct cytotoxic effects, UV light induces other mediators of cell death that promote KC death. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a potent inducer of apoptosis, and the TRAIL receptor R1 are expressed in lesions of CLE by KCs and infiltrating immune cells (Zahn et al. 2011). The UV-induced cytokine milieu and specifically type 1 interferons (IFNs) promote the expression of these molecules. TRAIL, in addition to inducing apoptosis, has a pro-inflammatory property such as the induction of IL-8 and intercellular adhesion molecules (such as ICAM-1). Likewise, overexpression of IL-18 receptor is noted in CLE KC in response to UV-induced cytokines TNF-α and IFN-γ. IL-18 signaling then promotes KC death (Wang et al. 2008).

In addition to KC death, increased neutrophil infiltration and death is noted in CLE (Villanueva et al. 2011). Neutrophil extracellular traps (NETs) are released by dying neutrophils and contain chromatin structures loaded with antimicrobial peptides (AMPs). These DNA-AMP complexes serve as B-cell auto-antigens promoting the generation of DNA- and RNA-specific autoantibodies that are characteristic of SLE. DNA-AMP complexes also stimulate plasmacytoid DC (pDC) to produce type 1 IFN through Toll-like receptor ligation (Craft 2011; Knight and Kaplan 2012). UV-induced IFN-α further primes local neutrophils for NETosis initiating a positive feedback loop. Reduced CD44 expression on neutrophils and the presence of DNAse inhibitors and anti-DNAse antibodies are possible explanations for the impaired clearance of dying neutrophils (Craft 2011).

UV-Mediated Promotion of Inflammation

UV stimulates KC to release multiple cytokines resulting in an inflammatory environment. UV light promotes KC to release TNF-α, IL-1 family (IL-1α, IL-1β, IL-18, IL-33), IL-8, IL-6, IFN-γ, granulocyte colony-stimulating factor (G-CSF), and, critically, type 1 interferons (IFN, mainly IFN-α) (Reefman et al. 2008).

Type 1 IFNs play a key role in innate immune responses and prime the immune system for adaptive immune responses. Type 1 IFNs modulate autoimmunity by promoting DC maturation, T-cell survival, B-cell differentiation, immunoglobulin class switching, and antibody production. Plasmacytoid DCs, which are the primary source for this cytokine, are detected in the skin of lupus patients but not in normal skin. The type 1 IFN-inducible protein MxA is abundant in CLE skin, suggesting local overproduction of this type 1 IFN. The overexpression of type 1 IFN-related

genes in the clinically normal skin of patients with SLE is consistent with a genetically determined activation of the pathway in these patients (Obermoser and Pascual 2010). Type III IFNs share many functions with type I IFN but are primarily expressed by and act upon epithelial cells. Expression of both type III IFN and type III INF receptors is enhanced in CLE (Zahn et al. 2011). Increased expression of epithelial-derived interferon may be an initial step in the initiation of the immune response on CLE.

UV irradiation induces IL-1 and TNF-α production by KCs. Further, in CLE PBMCs, TNF-α production is significantly elevated and correlates with disease activity (Nabatian et al. 2012). IL-1 stimulates the activation of a network of cytokines including chemokines and adhesion molecule upregulation. Finally, UV-induced IL-6 stimulates B-cell differentiation and immunoglobulin secretion and thus promotes autoantibody formation.

UV light also induces release of chaperonins like HMGB-1 from the cell nucleus to the extracellular milieu. In the extracellular milieu, HMGB-1 regulates cell migration and inflammatory responses and cytokine production such as type 1 IFN, TNF-α, and IL-1β. High expression of HMGB-1 has been shown in photo-provoked skin lesions in patients with CLE after UV irradiation, which coincided with CLE lesion induction (Barkauskaite et al. 2007). Finally, AMPs such as LL37 contribute to human skin defense, but these molecules may also participate in increased local release of type 1 IFN, promoting inflammation. AMPs are induced by UV and are expressed in CLE lesions (Kreuter et al. 2011).

Genetic Determinants of CLE

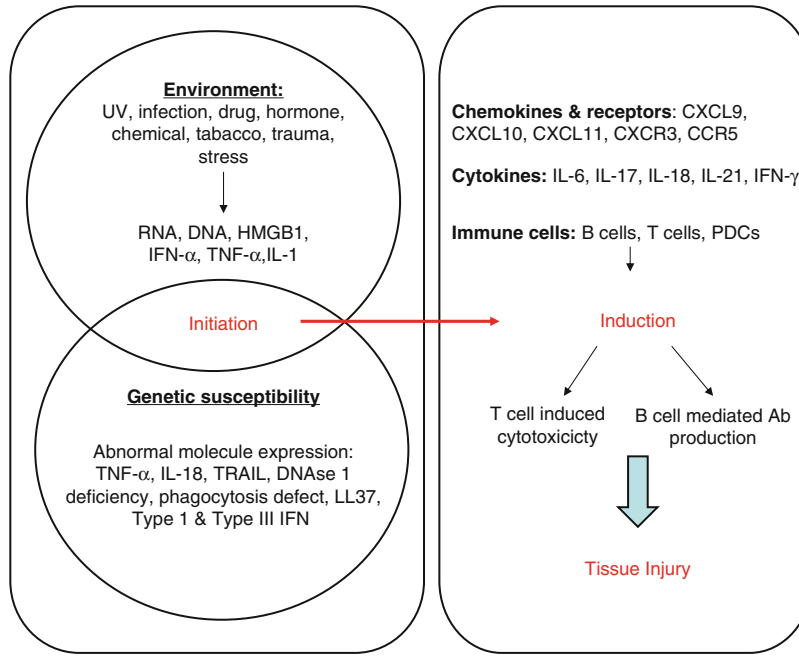
Subacute cutaneous lupus erythematosus (SCLE) is a distinct subtype of CLE that is characterized by exquisite photosensitivity and the development of polycyclic and annular lesions. This form of CLE is associated with antibodies to Ro/SSA and is linked to the HLA1, HLA-B8, DR-3, DRw52, and C4 null haplotypes in Caucasians. Other genes associated with CLE in large genome-wide studies include polymorphisms in ITGAM, IRF5, TYK2, and FCGR2A (reviewed in (Zhang 2012)). These genes are involved in type 1 IFN signaling and dead cell clearance.

Induction of Skin Injury in CLE

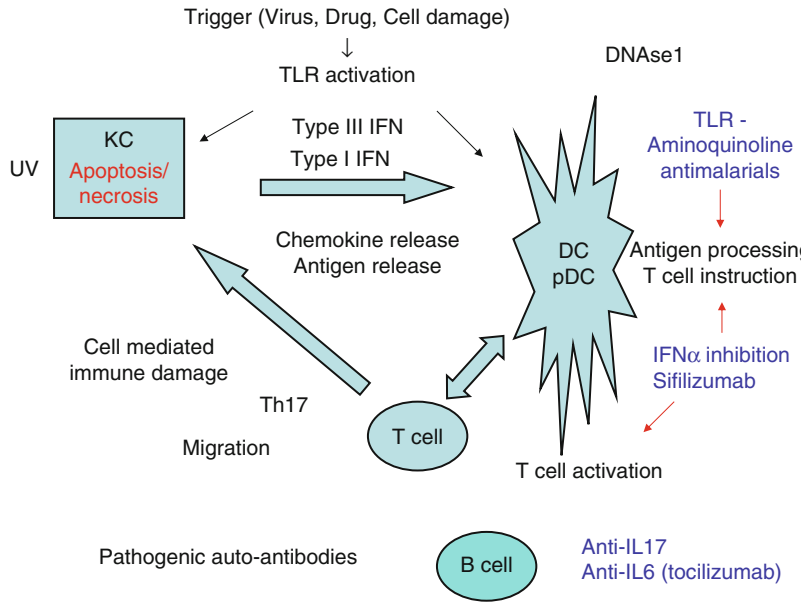
Activation and infiltration of inflammatory cells including T cells and B cells, DCs, pDC, and macrophages occurs in CLE skin, resulting in further damage to the skin. Thus, the pathology of CLE is a "lichenoid tissue reaction" where basal KCs are targeted by inflammatory cells, including granzyme-positive cytotoxic $CD8^+$ T cells and $CD4^+$ T cells (Dutz 2009; Sontheimer 2009). This tissue reaction depends upon the appropriate expression of immune homing molecules or chemokines resulting in the attraction and retention of damaging T cells (Meller et al. 2009). Upregulation of inflammatory CXCR3 ligands, CXCL9, CXCL10, and CXCL11, is associated with the recruitment of both effector cytotoxic T cells and pDC and is characteristic in CLE (Wenzel et al. 2009). Importantly, type 1 IFN signaling promotes a Th1-biased and Th17-biased inflammation. The Th1-associated chemokine receptor 5 (CCR5) is highly expressed by circulating T cell in patients with active CLE (Freutel et al. 2011). Elevated levels of Th17-type cytokine IL-17 are noted in the skin and serum of patients with CLE and SLE (Tanasescu et al. 2010). IL-21, a cytokine produced by activated CD4 T cells and NK cells, affects B and T cells, NK T cells, DCs, monocytes, and macrophages and generally promotes cytotoxicity. IL-21 is also elevated in lesional CLE (Costanzo et al. 2010).

Summary

In CLE, genetic susceptibilities promote the release, dislocation, or overexpression of molecules including DNA, RNA, HMGB1, TRAIL, and AMPs and the production of cytokines or chemokines including IL-1, IL-6, IL-10, IL-18, and TNF-α. Environmental factors such as



Lupus Erythematosus, Skin, Fig. 1 Summary depicting the proposed interaction between genetic predisposing factors and environment in the development of cutaneous lupus erythematosus lesions



Lupus Erythematosus, Skin, Fig. 2 Model of the pathogenesis of cutaneous lupus erythematosus. Skin cell (keratinocyte) damage is proposed to be the primary initiating event. The *thin red arrows* indicate potential novel therapeutic targets

UV are required to trigger lesion induction in susceptible individuals through the generation of inflammatory cells that induce immune-mediated damage at the initial trigger site. These interactions are depicted in Fig. 1.

Current therapies for CLE involve the use of topical anti-inflammatory drugs such as corticosteroids and calcineurin inhibitors. Aminoquinolone antimalarials are an effective treatment, possibly due to the inhibition of Toll-like receptor signaling (Kuhn et al. 2010). New therapies to "prevent" skin lesions formation should focus on targeting abnormally expressed or released molecules or should replace deficient

molecules combined with blocking the environmental triggering factor. Once lesions are developed, novel therapies should target aberrant immune response including targeting immune cells, autoantibodies, or cytokines to ameliorate the tissue damage. The potential points of therapy are depicted in Fig. 2.

Cross-References

- Chemokines
- Discoid Lupus
- Discoid SLE
- Environment and Autoimmunity
- Interleukin-6
- Skin in Systemic Lupus Erythematosus
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Barkauskaite V, Ek M, Popovic K, Harris HE, Wahren-Herlenius M, Nyberg F. Translocation of the novel cytokine HMGB1 to the cytoplasm and extracellular space coincides with the peak of clinical activity in experimentally UV-induced lesions of cutaneous lupus erythematosus. Lupus. 2007;16:794–802.

Bijl M, Reefman E, Limburg PC, Kallenberg CG. Inflammatory clearance of apoptotic cells after UVB challenge. Autoimmunity. 2007;40:244–8.

Costanzo A, Chimenti MS, Botti E, Caruso R, Sarra M, Monteleone G. IL-21 in the pathogenesis and treatment of skin diseases. J Dermatol Sci. 2010;60:61–6.

Craft JE. Dissecting the immune cell mayhem that drives lupus pathogenesis. Sci Transl Med. 2011;3:73ps9.

Dutz JP. T-cell-mediated injury to keratinocytes: insights from animal models of the lichenoid tissue reaction. J Invest Dermatol. 2009;129:309–14.

Freutel S, Gaffal E, Zahn S, Bieber T, Tuting T, Wenzel J. Enhanced CCR5+/CCR3+ T helper cell ratio in patients with active cutaneous lupus erythematosus. Lupus. 2011;20:1300–4.

Gaipl US, Munoz LE, Grossmayer G, Lauber K, Franz S, Sarter K, Voll RE, Winkler T, Kuhn A, Kalden J, Kern P, Herrmann M. Clearance deficiency and systemic lupus erythematosus (SLE). J Autoimmun. 2007;28:114–21.

Ghoreishi M, Dutz JP. Cutaneous lupus erythematosus: recent lessons from animal models. Lupus. 2010;19:1029–35.

Knight JS, Kaplan MJ. Lupus neutrophils: 'NET' gain in understanding lupus pathogenesis. Curr Opin Rheumatol. 2012;24:441–50.

Kreuter A, Jaouhar M, Skrygan M, Tigges C, Stucker M, Altmeyer P, Glaser R, Gambichler T. Expression of antimicrobial peptides in different subtypes of cutaneous lupus erythematosus. J Am Acad Dermatol. 2011;65:125–33.

Kuhn A, Ochsendorf F, Bonsmann G. Treatment of cutaneous lupus erythematosus. Lupus. 2010a;19:1125–36.

Kuhn A, Ruland V, Bonsmann G. Photosensitivity, phototesting, and photoprotection in cutaneous lupus erythematosus. Lupus. 2010b;19:1036–46.

Meller S, Gilliet M, Homey B. Chemokines in the pathogenesis of lichenoid tissue reactions. J Invest Dermatol. 2009;129:315–9.

Nabatian AS, Bashir MM, Wysocka M, Sharma M, Werth VP. Tumor necrosis factor alpha release in peripheral blood mononuclear cells of cutaneous lupus and dermatomyositis patients. Arthritis Res Ther. 2012;14:R1.

Obermoser G, Pascual V. The interferon-alpha signature of systemic lupus erythematosus. Lupus. 2010;19:1012–9.

Obermoser G, Sontheimer RD, Zelger B. Overview of common, rare and atypical manifestations of cutaneous lupus erythematosus and histopathological correlates. Lupus. 2010;19:1050–70.

Reefman E, Kuiper H, Limburg PC, Kallenberg CG, Bijl M. Type I interferons are involved in the development of ultraviolet B-induced inflammatory skin lesions in systemic lupus erythaematosus patients. Ann Rheum Dis. 2008;67:11–8.

Shao WH, Cohen PL. Disturbances of apoptotic cell clearance in systemic lupus erythematosus. Arthritis Res Ther. 2011;13:202.

Sontheimer RD. Lichenoid tissue reaction/interface dermatitis: clinical and histological perspectives. J Invest Dermatol. 2009;129:1088–99.

Tanasescu C, Balanescu E, Balanescu P, Olteanu R, Badea C, Grancea C, Vagu C, Bleotu C, Ardeleanu C, Georgescu A. IL-17 in cutaneous lupus erythematosus. Eur J Intern Med. 2010;21:202–7.

Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, Rubin CJ, Zhao W, Olsen SH, Klinker M, Shealy D, Denny MF, Plumas J, Chaperot L, Kretzler M, Bruce AT, Kaplan MJ. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol. 2011;187:538–52.

Wang D, Drenker M, Eiz-Vesper B, Werfel T, Wittmann M. Evidence for a pathogenetic role of interleukin-18 in cutaneous lupus erythematosus. Arthritis Rheum. 2008;58:3205–15.

Wenzel J, Zahn S, Bieber T, Tuting T. Type I interferon-associated cytotoxic inflammation in cutaneous lupus erythematosus. Arch Dermatol Res. 2009;301:83–6.

Wenzel J, Zahn S, Tuting T. Pathogenesis of cutaneous lupus erythematosus: common and different features in distinct subsets. Lupus. 2010;19:1020–8.
Zahn S, Rehkamper C, Ferring-Schmitt S, Bieber T, Tuting T, Wenzel J. Interferon-alpha stimulates TRAIL expression in human keratinocytes and peripheral blood mononuclear cells: implications for the pathogenesis of cutaneous lupus erythematosus. Br J Dermatol. 2011a;165:1118–23.
Zahn S, Rehkamper C, Kummerer BM, Ferring-Schmidt S, Bieber T, Tuting T, Wenzel J. Evidence for a pathophysiological role of keratinocyte-derived type III interferon (IFNlambda) in cutaneous lupus erythematosus. J Invest Dermatol. 2011b;131:133–40.
Zhang X. Genome-wide association study of skin complex diseases. J Dermatol Sci. 2012;66:89–97.

Lupus Nephritis and Novel Therapies, Pathogenesis

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Synonyms

Lupus; Nephritis; Pathogenesis; Systemic Lupus Erythematosus

Definition

Lupus nephritis (LN) is a common and often severe complication of Systemic Lupus Erythematosus (SLE). LN is defined as a manifestation of a systemic autoimmune disease characterized by loss of self-tolerance which results in immune complex accumulation in the kidney glomeruli. Despite extensive investigation, the pathogenesis of LN remains incompletely understood. In fact, LN is thought to be a heterogeneous disorder involving several pathogenic mechanisms. This entry will review the current understanding of the pathogenesis of LN and discuss novel therapeutic targets for treatment.

Introduction

SLE is an autoimmune, multisystem disease. It is characterized by the production of autoantibodies directed against nuclear and cellular antigens and the formation of pathogenic immune complexes. The exact pathogenesis of LN remains elusive. Multiple abnormalities of innate and adaptive immunity have been described, and immunologic dysfunction may precede clinical manifestations by several years. SLE and LN occur when there is a loss of tolerance to self-antigens with production of autoantibodies to these self-antigens. Normally, up to 75 % of newly formed and 40 % of mature B cells recognize self-antigens (Foster 2007). These produce natural IgM autoantibodies that are generally of low affinity for self-antigens. In SLE, however, autoantibodies undergo somatic mutation, class switching (IgM to IgG), and become high affinity and are directed against diverse epitopes on autoantigens (Foster 2007). The binding of autoantibodies to self-antigen creates immune complexes which can accumulate in the kidney activating inflammatory mediators such as complement, T cells, and macrophages which promote intrarenal inflammation leading to the development of LN. Hyper-reactive B and T cells, abnormal antigen presentation and increased levels of autoantigens, and defective clearance of apoptotic debris enhance production of pathogenic autoantibodies (Stuart and Hughes 2002).

Autoantibodies and LN

A necessary feature of the kidney biopsy in patients with LN is the presence of immuno globulins (pathologic features of LN on kidney biopsy can be found in the entry on

Lupus Nephritis and Novel Therapies, Pathogenesis, Table 1 Autoantibodies with pathogenic activity in lupus nephritis

Autoantibodies	Prevalence	Location
Anti-nucleosome	60–90 %	GBM, mesangial cells, endothelial cells, epithelial cells
Anti-dsDNA	70–96 %	GBM, mesangial cells, endothelial cells, epithelial cells, PTCs
Anti-Smith	10–60 %	GBM
Anti-Ro	25–44 %	GBM
Anti-C1Q	40–97 %	GBM, mesangial cells, TBM
Anti-ribosomal P protein	75 %	Glomerulus, mesangial cells
Anti-alpha-actinin	20 %	Glomerulus, mesangium, podocytes
Anti-Annexin II	32–65 %	Glomerulus, mesangium

GBM glomerular basement membrane, *PTC* peritubular capillaries, *TBM* tubular basement membrane

▶ Lupus Nephritis, Diagnosis and Treatment). Patients with active disease often demonstrate high-serum levels of circulating antinuclear and anti-double stranded (ds)DNA antibodies, the autoantibodies most associated with SLE. However, over 100 additional self-antigens have been identified in SLE as targets for autoantibodies (Kyttaris 2010), and several autoantibodies associated with LN are listed in Table 1. Autoantibodies to dsDNA and complement component C1q maybe particularly important in LN.

High-titer anti-dsDNA antibodies have been found to be associated with active LN (Tsao et al. 1992; Vlahakos et al. 1992). Anti-dsDNA antibodies are predominantly IgG subclasses 1 and 3 which are the most proinflammatory IgG subclasses due to their ability to activate complement and engage Fc receptors for IgG (Manolova et al. 2002). The classical view of LN pathogenesis is that circulating immune complexes deposit in the kidney and initiate an intrarenal inflammatory process through these mechanisms. Experimental work suggests that anti-dsDNA antibodies directly cross-react with glomerular antigens such as mesangial cell membranes, laminin, α-actinin, and exposed chromatin fragments on glomerular basement membranes (Madaio et al. 1987). Glomerular antigens may be exposed due to defective clearance of apoptotic debris. As an example, DNase 1 may be deficient in SLE exposing chromatin for binding to anti-dsDNA antibodies (Mortensen and Rekvig 2009). Furthermore, defective clearance of apoptotic debris such as chromatin can activate intrarenal dendritic cells, enhance T-B cell interaction, and increase production of anti-chromatin antibodies (Mortensen and Rekvig 2009). Supporting this theory is the identification of nucleosome specific T cells and induction of anti-nucleosome, anti-dsDNA, and anti-histone antibodies through Th cells (van der Vlag and Berden 2011). These antibodies have been detected in both lupus mice and in patients with SLE.

C1q antibodies have been strongly correlated to lupus nephritis (Sinico et al. 2005; Meyer et al. 2009). C1q is thought to be a crucial participant in macrophage-mediated clearance of apoptotic material which is important in regulating the development of autoreactive B cells. Both mice and humans with C1q deficiency are characterized by an autoimmune phenotype similar to SLE (Stoll and Gavalchin 2000). Thus, C1q has an important role in maintaining self-tolerance. Additionally, anti-C1q antibodies trigger classical complement pathway amplification enhancing renal tissue damage. Patients with active LN demonstrate high levels of circulating anti-C1q antibodies as well as deposition in the renal biopsy tissue. The absence of anti-C1q antibodies has a high negative predictive value for active LN (Mok et al. 2010), while its presence may be a potential marker for disease activity (Tsirogianni et al. 2009).

Genetics in LN

Genetics also play a role in LN. Using genome-wide and candidate gene studies, over 30 genes

have been identified that appear to be involved in the pathogenesis of SLE in general (Tsao 2004). These include immune-complex clearance genes, immune response genes, and IFN-α signaling and response genes. The specific genetics of human LN are less clear. Studies in LN suggest significant heterogeneity in different racial/ethnic groups and are therefore challenging. Genes that seem to play a role in LN include those for Fc receptors for IgG, the cytokines monocyte chemotactic protein-1 (MCP-1) and interleukin (IL)-18, signal transducer and activator of transcription-4 (STAT4), and the HLA DR3 allele (DRB1 *0301) (Vuong et al. 2010).

The Fc receptor is of particular importance in the pathogenesis of LN as Fc receptors for IgG (known as Fcγ receptors, or FcγR) are engaged by immune complexes and mediate either protection or injury. Protection is facilitated by FcγR-induced phagocytosis and clearance of the immune complexes. Inflammation is facilitated because the FcγR engaged by immune complexes activate their leukocytes to secrete proinflammatory molecules such as oxygen free radicals and proteolytic enzymes (Waldman and Madaio 2005). FcγR with a genetically determined lower affinity for immune complexes have been associated with SLE and particularly with LN. This suggests that forms of FcγR that bind more efficiently promote immune complex clearance, and lower affinity FcγR promote activation of proinflammatory leukocytes (Clatworthy et al. 2007) (Foster 2007).

Complement and LN

The role of complement in LN is related to its affinity for immune complexes. The complement cascade ideally removes circulating immune complexes before they can mediate injury. The complement system is activated by circulating immune complexes and either solubilizes immune complexes so they are less likely to become trapped in tissue or opsonises them through complement activation products C4b and C3b/bi for direct clearance. Additionally, complement functions to remove apoptotic debris through opsonization by complement component C1q. However, once immune complexes have deposited in tissue, complement can drive tissue inflammation and can directly injure cells via the membrane attack complex or indirectly through activation of proinflammatory cytokines and toxic mediators (Bao and Quigg 2007). For further detail, please see the entry ▶ Complement Regulation in the Kidney located elsewhere in the Encyclopedia.

T and B cells in LN

Kidney-infiltrating T cells play several roles in LN. These include T cells providing help to B cells, production of autoantibodies through T cell effector functions, generation of cytokines, and direct infiltration into renal tissue triggering inflammation through recruitment of proinflammatory leukocytes, including macrophages.

Abnormalities in both Th1 and Th2 cytokines occur in LN; however, intrarenal production of Th1 cytokines, specifically IL-12, IFN-γ, and IL-18, appears to exceed Th2 cytokines in proliferative LN and correlates with histologic activity (Masutani et al. 2001). Th1 responses are associated with activated macrophages and with the production of immunoglobulin isotypes capable of activating complement and FcγR pathways. The Th1 dominance displayed in LN patients, both locally in the kidney and systemically in the circulation, suggests that this may be an important prerequisite for developing LN.

MRL/lpr mice spontaneously develop a severe autoimmune syndrome closely resembling human SLE and have been shown to develop severe nephritis due to polyclonal expansion of $CD4^+$ T cells suggesting a pathogenic role in promoting nephritis (Okamoto et al. 2012). $CD4^+$ T cells have been implicated in the development of nephritis in experimental lupus models with several co-stimulatory interactions playing important roles. Interruption of co-stimulatory pathways, including CD28/B7 and CD40/CD40L, attenuates renal injury in animal models (Peng 2004).

IFN-γ is also thought to be pathogenic in murine LN models. Lupus-prone BWF1 mice develop nephritis after administration of IFN-γ,

while renal disease was prevented in murine LN models after the administration of neutralizing antibodies and in those deficient in the IFN-γ receptor (Foster 2007). Several other cytokines including IL-6, IL-12, IL-23, IL-27, and TGF-beta have been shown to affect the kidney through various mechanisms in murine models of SLE (Mishra et al. 2003; Kelley and Wuthrich 1999).

Human regulatory T cells, characterized as $CD4^+CD25^+$, inhibit immune responses through effects on T and B cells, and particularly autoantibody production. Patients with SLE have normal and reduced number of regulatory T cells that are functionally defective during disease activity. Additionally, regulatory T cells have a protective effect in lupus-prone mice, and mice depleted of regulatory T cells develop accelerated autoimmune features (Foster 2007; Tucci et al. 2010).

Like T cells, B Cells are also heavily involved in the pathogenesis of LN. Abnormalities in B cell tolerance allows for the development of autoantibodies and eventual immune complex deposition in the kidney. In the MRL/lpr model, B cell-deficient mice are protected from disease, whereas those mice with nonsecretory plasma cells still develop autoimmunity, suggesting B cell involvement beyond antibody production (Foster 1999). In SLE, there is impaired removal of autoreactive B cells. B cells are also efficient antigen-presenting cells (APC) that can process and present antigens to T cells via MHC Class I and MHC Class II. Dysregulation of apoptotic cell clearance results in exposed nuclear antigens on cells surfaces and results in the activation of autoreactive B cells and presentation of antigens to autoreactive T cells. This may directly contribute to intrarenal inflammation. Additionally, chemoattractants secreted from B cells can influence the chemotaxis of other immune cells such as T cells and monocyte-derived dendritic cells (Chang et al. 2011).

Toll-Like Receptors and LN

Toll-like receptors (TLR) are present on cell membranes (TLR2 with TLR1 or 6, TLR4, TLR5, TLR11) and endosomal membranes (TLR3, TLR7/TLR8, TLR9, TLR10) in various cell types. Several types of TLRs have been identified in humans. They are able to recognize bacterial, viral, and fungal antigens as well as endogenous ligands. Ligand binding to TLRs activate Type I IFN production and/or proinflammatory cytokine production (Conti et al. 2011).

TLRs have been demonstrated to have a role in LN in both human and animal studies. Mice overexpressing TLR7 have premature mortality with the increased production of proinflammatory cytokines. TLR7 has a role in the activation of autoreactive B cells which are directed against RNA-associated autoantigens. In the MRL/lpr animal model, the presence of TLR7 ligands increased the development of glomerulonephritis, while a lack of TLR7 decreased the severity of renal involvement (Santiago-Raber et al. 2009).

In lupus-prone animal models, deletion of TLR9 decreases the production of anti-nucleosome/histone antibodies in one model and the production of anti-DNA autoantibodies in another model. Experiments with transgenic mice have shown that the TLR9 signaling pathway is crucial in the generation of pathogenic anti-DNA/polyreactive antibodies. However, there have been reports that in TLR9-/- lupus-prone mice (MRL-, $B6\text{-}Fas^{lpr}$), nephritis still developed despite the absence of anti-dsDNA antibodies (Santiago-Raber et al. 2009). This suggests that reactivity of autoantibodies to DNA or chromatin may not be necessary, but instead the ability of autoantibody to react with antigen on renal parenchymal cells could drive disease (Christensen et al. 2005).

Immune complexes containing DNA and RNA are endogenous ligands for both TLR7 and TLR9, and activation of these intracellular TLRs induces plasmacytoid dendritic cell IFN-α production. IFN-α is an important cytokine in SLE and LN, with the serum level of INF-α as well as expression of IFN-α-dependant genes in monocytes correlating with disease activity (Anders et al. 2010). The effects of IFN-α on the immune response include promoting maturation of conventional dendritic cells into potent antigen-presenting cells, inducing B cell differentiation to plasma cells, and contributing to the development of CD4 helper and CD8 memory T cells.

Neutrophils and Neutrophil Extracellular Traps (NETs)

Neutrophils are also now being recognized as important participants in the pathogenesis of SLE. Lupus neutrophils are thought to be of lower density, with decreased phagocytic and lysosomal activity compared to normal neutrophils. These Low-Density Granulocytes (LDGs) are proinflammatory and cause direct tissue damage. Peptides released from LDGs are toxic to glomerular structures, and in experimental models, depletion of neutrophils protects against antibody-mediated glomerulonephritis (Knight and Kaplan 2012). NETosis is a form of neutrophil cell death. NETs are chromatin structures that bind foreign material including microbes and in SLE, autoantigens. In SLE, aberrant NETosis leads to the potent release of NETs from LDGs stimulating IFN-α secretion by dendritic cells and causing direct tissue damage through toxic mediators. This occurs through a complex interaction between DNA, anti-DNA autoantibodies, and neutrophil-derived peptide LL37. The role of LL37 is not entirely clear; however, it is thought to stabilize NETs and prevent degradation by DNase1. This perpetuates a cycle of continuous autoreactivity driven by IFN-α and continuous tissue injury by NETs (Garcia-Romo et al. 2011; Lande et al. 2011; Knight and Kaplan 2012).

Biomarkers for LN

There is considerable interest in identifying biomarkers that reflect impending LN flare, disease severity and that can predict response to therapy and disease progression. Several urine biomarker candidates have been identified, but to date none have been validated for clinical use in a large, prospectively followed lupus cohort. The rationale for developing LN biomarkers is to improve real-time disease management and achieve more complete renal remissions. A few potential biomarkers are described below.

Monocyte chemoattractant protein 1 (MCP-1) has been shown to be involved in the pathogenesis of lupus nephritis in both animal and human studies. Urine MCP-1 levels are higher in active renal disease than healthy controls or those with nonrenal SLE or inactive LN. Levels are also higher in proliferative LN compared to non-proliferative LN. However, urinary MCP-1 is also elevated in several other glomerulonephritides and is not specific for LN (Rovin and Zhang 2009).

Another biomarker of interest is neutrophil gelatinase-associated lipocalin (NGAL). NGAL is involved in cellular iron transport and is constitutively expressed within the kidney. An increase in urinary NGAL is predictive of acute kidney injury with both serum and urine levels correlate better to glomerular filtration rate than more conventional analytes such as serum creatinine. NGAL has been shown to be sensitive and specific for active LN in children, with levels increasing prior to clinical flare. However, similar to MCP-1, elevated levels of urine NGAL are also seen in other types of renal injury (Han et al. 2009).

Novel Therapies for Lupus Nephritis

Cytotoxic therapies have improved outcomes in LN; however, current standard therapies such as cyclophosphamide are nonspecific and associated with significant adverse effects. The need for new approaches for the treatment of LN is also highlighted by the low complete and modest overall remission rate with current approaches. As more is understood regarding the immunopathology of LN, drugs with more targeted mechanisms of action can be developed. Several targeted LN therapies are currently being evaluated in clinical trials.

Rituximab is a chimeric mouse/human monoclonal antibody targeted against CD20. CD20 is present on B cells from the pre-lymphocyte stage to more mature lymphocytes. As described above, B cells play an important role in the pathogenesis of lupus, and rituximab is able to deplete B cells through complement-mediated cell lysis and Fc receptor-mediated cellular cytotoxicity and by inducing apoptosis (Maloney et al. 2002).

Fcγ-RIIIa genotype may help predict the level of B cell depletion to rituximab therapy. Specific variants of the Fcγ-RIIIa gene, described above, can limit rituximab efficacy in SLE and may be an important tool to guide clinicians in the future (Anolik et al. 2003). Additional effects of rituximab include increasing regulatory T cells, decreasing the T cell co-stimulatory molecule CD40 ligand, and decreasing other important co-stimulatory molecules (Vigna-Perez et al. 2006). A potential complication associated with rituximab use is autoantibody formation against rituximab. Formation of these human anti-chimeric antibodies (HACA) is thought to be more prevalent in SLE than in other diseases treated by rituximab. The effect of HACA is reduced rituximab-mediated B cell depletion, loss of rituximab, and serum sickness. Premedication with intravenous steroids reduces the incidence of antibody development (Saito et al. 2005).

Epratuzumab is a humanized monoclonal antibody directed against CD22, which is expressed on the surface of mature B cells. The mechanism of action is through the B cell receptor and modulation of B cell responses. Epratuzumab has been shown to be effective in moderate to severe SLE but has not yet been evaluated in human LN.

B-lymphocyte stimulator protein (BLyS) is a member of the TNF superfamily and is an important cytokine in the development and survival of B cells through interaction with 3 different receptors. Elevated BLyS levels have been demonstrated in patients with SLE and are associated with disease activity. Belimumab is a fully humanized monoclonal antibody specific for BLyS and inhibits its activity. A recent phase III trial demonstrated safety and efficacy of Belimumab in treatment of SLE, although active LN patients were specifically excluded (Navarra et al. 2011).

The interaction and signaling between B cell and T cells is crucial in the pathogenesis of lupus, and these co-stimulatory molecules have become targets for immunotherapy. CD28 is expressed on resting T cells and once T cells have been activated CTLA4 is expressed. These molecules are able to bind to receptors, CD80(B7.1)/CD86(B7.2), which are on antigen-presenting cells. CTLA4, once bound to these receptors, inhibits the T cell response and therefore limits T cell proliferation. Abatacept is a fusion protein between the extracellular domain of CTLA4 and the Fc portion of IgG (CTLA4-Ig) that is able to function as a receptor for B7, and this inhibits T cell activation. In murine LN, CTLA4-Ig was able to decrease proteinuria, autoantibody production, and mortality. There is now a clinical trial underway investigating the use of abatacept as adjunct therapy in treatment of LN.

Another cytokine being targeted in LN is IL6. High levels of IL6 have been demonstrated in patients with SLE, and the administration of a monoclonal antibody against IL6 in LN mice resulted in decreased proteinuria and increased survival (Cordeiro and Isenberg 2008; Karim et al. 2009). Currently, a study evaluating the safety and effectiveness of an anti-IL-6 antibody in LN is underway.

Finally, TNF-like weak inducer of apoptosis (TWEAK) is a member of the TNF superfamily and regulates cell proliferation, cell death, and inflammation through interaction with the fibroblast growth factor-inducible 14 receptor (fn14). Current evidence suggests TWEAK may be a target in renal and vascular injury. In SLE, urinary TWEAK levels are elevated during acute LN flares and the TWEAK-fn14 pathway may be involved in the pathogenesis of LN (Schwartz et al. 2009). A randomized clinical trial is underway to assess the efficacy of anti-TWEAK as an adjunct therapy for LN.

Summary

The exact pathogenesis of LN remains unclear and significant efforts are being made to identify the multiple mechanisms involved in this heterogenous disorder. LN occurs due to a loss of self-tolerance leading to autoantibody formation and immune complex accumulation in the kidney, triggering a multifaceted immune reaction that involves activation of complement, T cells, B cells, and macrophages. Intra-renal

inflammation follows and leads to the development of LN. As more is learned about the pathogenesis of LN, biomarkers to identify early and severe disease will be developed. Current approaches to treatment are suboptimal and new therapeutic approaches are being evaluated to provide more targeted therapy and improve outcomes while limiting side effects. A more clear understanding of the pathogenesis of SLE and LN is necessary to achieve these therapeutic goals.

Cross-References

- ▶ Complement Regulation in the Kidney
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Nephritogenic Antibodies in Systemic Lupus Erythematosus
- ▶ Novel Targets in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Pathogenesis

References

Anders HJ, Lichtnekert J, et al. Interferon-alpha and -beta in kidney inflammation. Kidney Int. 2010;77(10):848–54.

Anolik JH, Campbell D, et al. The relationship of FcgammaRIIIa genotype to degree of B cell depletion by rituximab in the treatment of systemic lupus erythematosus. Arthritis Rheum. 2003;48(2):455–9.

Bao L, Quigg RJ. Complement in lupus nephritis: the good, the bad, and the unknown. Semin Nephrol. 2007;27(1):69–80.

Chang A, Henderson SG, et al. In situ B cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. J Immunol. 2011;186(3):1849–60.

Christensen SR, Kashgarian M, et al. Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. J Exp Med. 2005;202(2):321–31.

Clatworthy MR, Willcocks L, et al. Systemic lupus erythematosus-associated defects in the inhibitory receptor FcgammaRIIb reduce susceptibility to malaria. Proc Natl Acad Sci USA. 2007;104(17):7169–74.

Conti F, Spinelli FR, et al. Toll-like receptors and lupus nephritis. Clin Rev Allergy Immunol. 2011;40(3):192–8.

Cordeiro AC, Isenberg DA. Novel therapies in lupus - focus on nephritis. Acta Reumatol Port. 2008;33(2):157–69.

Foster MH. Relevance of systemic lupus erythematosus nephritis animal models to human disease. Semin Nephrol. 1999;19(1):12–24.

Foster MH. T cells and B cells in lupus nephritis. Semin Nephrol. 2007;27(1):47–58.

Garcia-Romo GS, Caielli S et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med. 2011; 3(73): 73ra20

Han WK, Wagener G, et al. Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. Clin J Am Soc Nephrol. 2009;4(5):873–82.

Karim MY, Pisoni CN, et al. Update on immunotherapy for systemic lupus erythematosus–what's hot and what's not! Rheumatology (Oxford). 2009;48(4):332–41.

Kelley VR, Wuthrich RP. Cytokines in the pathogenesis of systemic lupus erythematosus. Semin Nephrol. 1999;19(1):57–66.

Knight JS, Kaplan MJ. Lupus neutrophils: 'NET' gain in understanding lupus pathogenesis. Curr Opin Rheumatol. 2012;24(5):441–50.

Kyttaris VC. Systemic lupus erythematosus: from genes to organ damage. Methods Mol Biol. 2010;662:265–83.

Lande R, Ganguly D et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. Sci Transl Med. 2011; 3(73): 73ra19

Madaio MP, Carlson J, et al. Murine monoclonal anti-DNA antibodies bind directly to glomerular antigens and form immune deposits. J Immunol. 1987;138(9):2883–9.

Maloney DG, Smith B, et al. Rituximab: mechanism of action and resistance. Semin Oncol. 2002;29(1 Suppl 2):2–9.

Manolova I, Dancheva M, et al. Predominance of IgG1 and IgG3 subclasses of autoantibodies to neutrophil cytoplasmic antigens in patients with systemic lupus erythematosus. Rheumatol Int. 2002;21(6):227–33.

Masutani K, Akahoshi M, et al. Predominance of Th1 immune response in diffuse proliferative lupus nephritis. Arthritis Rheum. 2001;44(9):2097–106.

Meyer OC, Nicaise-Roland P, et al. Anti-C1q antibodies antedate patent active glomerulonephritis in patients with systemic lupus erythematosus. Arthritis Res Ther. 2009;11(3):R87.

Mishra N, Reilly CM, et al. Histone deacetylase inhibitors modulate renal disease in the MRL-lpr/lpr mouse. J Clin Invest. 2003;111(4):539–52.

Mok CC, Ho LY, et al. Performance of anti-C1q, antinucleosome, and anti-dsDNA antibodies for detecting concurrent disease activity of systemic lupus erythematosus. Transl Res. 2010;156(6):320–5.

Mortensen ES, Rekvig OP. Nephritogenic potential of anti-DNA antibodies against necrotic nucleosomes. J Am Soc Nephrol. 2009;20(4):696–704.

Navarra SV, Guzman RM, et al. Efficacy and safety of belimumab in patients with active systemic lupus

erythematosus: a randomised, placebo-controlled, phase 3 trial. Lancet. 2011;377(9767):721–31.
Okamoto A, Fujio K, et al. Kidney-infiltrating CD4(+) T-cell clones promote nephritis in lupus-prone mice. Kidney Int. 2012;82(9):969–79.
Peng SL. Experimental use of murine lupus models. Methods Mol Med. 2004;102:227–72.
Rovin BH, Zhang X. Biomarkers for lupus nephritis: the quest continues. Clin J Am Soc Nephrol. 2009;4(11):1858–65.
Saito K, Nawata M, et al. Extremely high titer of anti-human chimeric antibody following re-treatment with rituximab in a patient with active systemic lupus erythematosus. Rheumatology (Oxford). 2005; 44(11):1462–4.
Santiago-Raber ML, Baudino L, et al. Emerging roles of TLR7 and TLR9 in murine SLE. J Autoimmun. 2009;33(3–4):231–8.
Schwartz N, Rubinstein T, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther. 2009;11(5):R143.
Sinico RA, Radice A, et al. Anti-C1q autoantibodies in lupus nephritis: prevalence and clinical significance. Ann N Y Acad Sci. 2005;1050:193–200.
Stoll ML, Gavalchin J. Systemic lupus erythematosus-messages from experimental models. Rheumatology (Oxford). 2000;39(1):18–27.
Stuart L, Hughes J. Apoptosis and autoimmunity. Nephrol Dial Transplant. 2002;17(5):697–700.
Tsao BP. Update on human systemic lupus erythematosus genetics. Curr Opin Rheumatol. 2004;16(5):513–21.
Tsao BP, Ohnishi K, et al. Failed self-tolerance and autoimmunity in IgG anti-DNA transgenic mice. J Immunol. 1992;149(1):350–8.
Tsirogianni A, Pipi E, et al. Relevance of anti-C1q autoantibodies to lupus nephritis. Ann N Y Acad Sci. 2009;1173:243–51.
Tucci M, Stucci S, et al. Cytokine overproduction, T-cell activation, and defective T-regulatory functions promote nephritis in systemic lupus erythematosus. J Biomed Biotechnol. 2010;2010:457146.
van der Vlag J, Berden JH. Lupus nephritis: role of antinucleosome autoantibodies. Semin Nephrol. 2011;31(4):376–89.
Vigna-Perez M, Hernandez-Castro B, et al. Clinical and immunological effects of Rituximab in patients with lupus nephritis refractory to conventional therapy: a pilot study. Arthritis Res Ther. 2006;8(3):R83.
Vlahakos DV, Foster MH, et al. Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. Kidney Int. 1992;41(6):1690–700.
Vuong MT, Gunnarsson I, et al. Genetic risk factors in lupus nephritis and IgA nephropathy–no support of an overlap. PLoS One. 2010;5(5):e10559.
Waldman M, Madaio MP. Pathogenic autoantibodies in lupus nephritis. Lupus. 2005;14(1):19–24.

Lupus Nephritis, Diagnosis and Treatment

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Synonyms

Lupus; Lupus nephritis; SLE; Systemic lupus erythematosus

Definition

The kidney is one of the major target organs affected by systemic lupus erythematosus (SLE). Lupus nephritis (LN) is the most common form of kidney disease in SLE, and occurs as a result of immune complex accumulation in the renal glomeruli. Depending on their location within glomeruli, immune complexes can trigger renal cell proliferation, matrix formation, or inflammation. Clinically, intra-renal immune complexes can manifest as hematuria, proteinuria, or kidney insufficiency. Although there are other types of kidney injury in SLE, this essay will focus on LN.

Introduction

Kidney involvement in SLE is common and often severe. Approximately 40 % of adults and 80 % of children with SLE develop clinically significant kidney disease, and kidney disease significantly increases the morbidity and mortality of SLE (Contreras et al. 2006; Saxena et al. 2011). Younger age (<33 years old), male sex, and non-European ancestry are thought to

be risk factors for development of LN (Saxena et al. 2011). Death attributable to renal disease occurs in 5–25 % of patients with proliferative LN within 5 years (Franco et al. 2010). The incidence of LN and progressive kidney failure is higher in Black, Hispanic, and Asian populations (Seligman et al. 2002). In fact, the progression to ESRD has been reported as high as nine times greater in Black patients (Korbet et al. 2007).

The diagnosis and prognosis of LN is confirmed by kidney biopsy and the histopathologic classification based on biopsy findings guides treatment. Current strategies for LN treatment use intense, nonspecific immunosuppressive therapy that carries considerable risk and often suboptimal results.

Diagnosis of Lupus Nephritis

Most patients with LN lack overt signs of kidney disease, but rather have abnormalities of serum creatinine and/or the urine sediment. Assessment of serum creatinine, urine dipstick and sediment, and quantification of proteinuria are important screening tools. While many patients will have findings of LN at initial diagnosis of SLE, all patients with SLE should undergo testing for LN at regular intervals because preservation of kidney function is best achieved by early diagnosis and treatment.

The urine dipstick is commonly used to screen for LN, and a dipstick positive for blood and protein is suggestive, but there is a high false-negative rate (Woolhandler et al. 1989). Microscopic examination of the urine sediment after centrifugation is thus essential to look for evidence of glomerular bleeding (dysmorphic red blood cells, acanthocytes, red blood cell casts) or renal inflammation (white blood cells, white blood cell casts in the absence of infection) (Image 1). The evaluate of urine sediment is discussed in detail elsewhere in this Encyclopedia, in the entry on "Urinalysis."

Proteinuria is the hallmark of LN, and in a comprehensive review 100 % of LN patients presented with proteinuria (Cameron 1999). Proteinuria is a biomarker for LN activity and is used to assess relapse, remission, and effect of treatment. Proteinuria is not simply a marker of disease but is also toxic to the kidney, and can cause progressive renal damage. The ideal method to quantify proteinuria is a 24-h urine collection for protein and creatinine (Parikh et al. 2011). An intended 24-h collection that is at least 50 % complete is also accurate. Measurement of the protein to creatinine ratio on the intended 24 h urine removes errors that may occur with under- or over-collections (Birmingham et al. 2007).

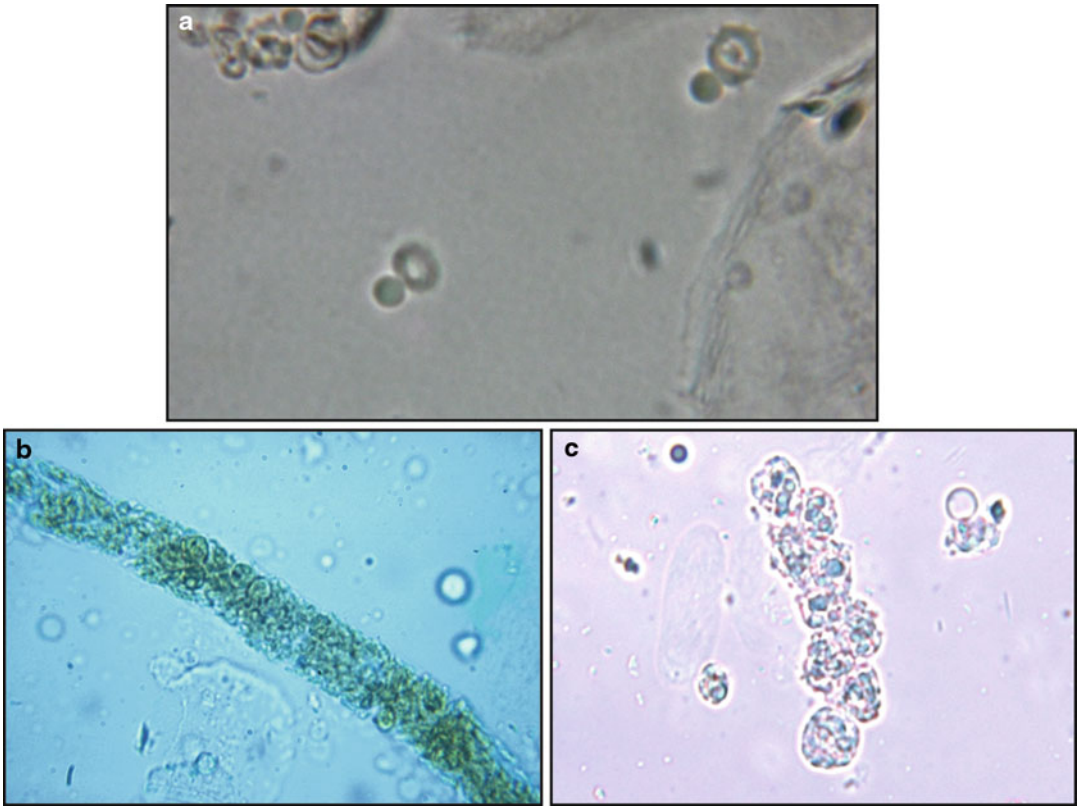
A kidney biopsy is the gold standard for diagnosis and classification of LN. A biopsy may not be necessary if the only clinical abnormalities suggesting LN are isolated hematuria, or minor proteinuria (<500 mg/day) in the absence of hematuria. A biopsy should be considered when proteinuria is over 500 mg/day, because this degree of proteinuria can be associated with significant kidney injury (Ardoin et al. 2011).

The Pathology of Lupus Nephritis

LN is currently classified by the 2003 International Society of Nephrology and the Renal Pathology Society (ISN/RPS) criteria (Table 1). These criteria define six classes of LN, and categorize renal lesions as active (A), chronic (C), or both, and whether they affect less than (segmental) or more than (global) 50 % of the glomerular surface area (Markowitz and D'Agati 2007).

Light microscopy describes active glomerular lesions as mesangial and endocapillary hypercellularity with associated capillary lumina occlusion and possible rupture of the capillary basement membranes leading to extracapillary cell proliferation and leukocyte infiltration (crescent formation). Chronic glomerular lesions include glomerular sclerosis and scarring (fibrosis). LN is classified as focal if <50 % of all glomeruli are injured, or diffuse if ≥50 % of all glomeruli are injured.

Immunofluorescence microscopy classically shows a "full house" pattern, which refers to the presence of IgG, IgA, IgM, C1q, C3, and kappa and lambda light chains all together in glomeruli. C1q deposition is often prominent and is specific



Lupus Nephritis, Diagnosis and Treatment, Image 1 *Urine sediment*: Urine microscopic examination demonstrating: (**a**) Acanthocytes – ring-shaped red cells with vesicle shaped protrusions or blebs that are indicative of glomerular bleeding. (**b**) Red blood cell (RBC) cast – urinary cast composed of a matrix containing RBCs and indicative of glomerular bleeding. (**c**) White blood cell (WBC) cast – urinary cast composed of matrix with WBCs and indicative of renal inflammation. For more detail, refer to entry on "Urinalysis"

for LN (Trendelenburg et al. 2006). It is uncommon to see full house pattern in other types of glomerulonephritis.

Ultrastructural evaluation of kidney biopsies by electron microscopy always shows mesangial electron-dense deposits, which are immune complexes. Outside the mesangium, immune deposits are variable in location. Subendothelial deposits appear between the glomerular capillary endothelial cells and the glomerular basement membrane, and are usually seen in proliferative LN. Subepithelial deposits are found between glomerular epithelial cells (podocytes) and the outer layer of the glomerular basement membrane, and are found in non-proliferative, membranous LN. Electron microscopy also often shows tubuloreticular structures in renal endothelial cells which are thought to be induced by interferon in lupus patients (Venkataseshan et al. 1991).

The specific classes of LN are:

Mesangial Lupus Nephritis (Class I and II)

Class I LN has normal glomeruli by light microscopy, but immunofluorescence and electron microscopy show mesangial immune complexes, a finding in all classes of LN. Class I LN typically has no overt clinical kidney abnormalities, so it is rarely diagnosed.

Class II, or mesangial proliferative LN, demonstrates pure mesangial hypercellularity and mesangial matrix expansion by light microscopy (Image 2), with mesangial immune deposits

Lupus Nephritis, Diagnosis and Treatment, Table 1 Lupus nephritis 2003 ISN/RPS classification

LN Class	Pathologic description	Treatment recommendations
Class I	Normal glomeruli by LM[a], mesangial immune complexes on IF[b]or EM[c]	IS[d] to treat extra-renal disease only
Class II	Pure mesangial hypercellularity with mesangial immune deposits. Mesangial matrix expansion seen by LM	IS to treat extra-renal disease only
		Renoprotective measures[e]
Class III	Active: segmental or global endo- and/or extracapillary proliferation in <50 % of glomeruli; subendothelial immune deposits	Active or active+chronic lesions – induction[f] and maintenance[g] IS
	Chronic: segmental or global glomerulosclerosis in <50 % of glomeruli	Chronic lesions only – renoprotective measures; IS to treat extra-renal disease
Class IV	Active: segmental or global endo- and/or extracapillary proliferation in ≥50 % of glomeruli; subendothelial immune deposits	Active or active+chronic lesions – induction[f] and maintenance[g] IS
	Chronic: segmental or global glomerulosclerosis in <50 % of glomeruli	Chronic lesions only – renoprotective measures; IS to treat extra-renal disease
Class V	Thickening of the glomerular basement membrane with global or segmental subepithelial immune deposits. May occur in combination of Class III or IV	Subnephrotic proteinuria – renoprotective measures. IS to treat extra-renal disease
		Nephrotic or abnormal renal function – treat with IS
Class VI	Advanced sclerosis ≥90 % of glomeruli are sclerosed	Renoprotective measures only

[a]LM – Light microscopy
[b]IF – Immunofluorescence
[c]EM – Electron microscopy
[d]IS – immunosuppression
[e]Renoprotective measures – Used for all patients with any form of LN. Includes anti-proteinuric therapy with blockade of renin-angiotensin-aldosterone system, blood pressure control, sodium restriction, protein restriction, and correction of metabolic abnormalities
[f]Induction therapy – IV or Oral cyclophosphamide or Mycophenolate mofetil for 3–6 months
[g]Maintenance therapy – Azathioprine or Mycophenolate mofetil after induction therapy to achieve and maintain remission; generally continued for at least 1 year after complete remission or indefinitely in partial remission

by immunofluorescence and electron microscopy. Clinically Class II LN shows microscopic hematuria and low-grade proteinuria, but kidney function is usually normal.

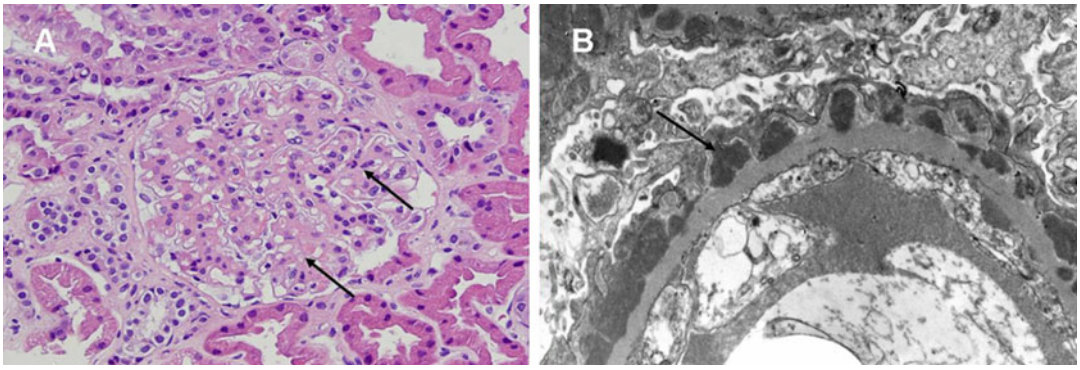
Proliferative Lupus Nephritis (Class III and IV)

Class III LN, also called focal proliferative LN, is characterized by endocapillary proliferation, crescents (Image 3), or both in fewer than 50 % of glomeruli, generally in a segmental distribution. Immunofluorescence and electron microscopy reveal mesangial and glomerular capillary immune complexes that localize to the subendothelial space. Lesions are further characterized as active, chronic, or having both chronic and active elements. Clinical evaluation reveals hematuria, proteinuria that may be in the nephrotic range (over 3.5 g/day), and normal or impaired kidney function.

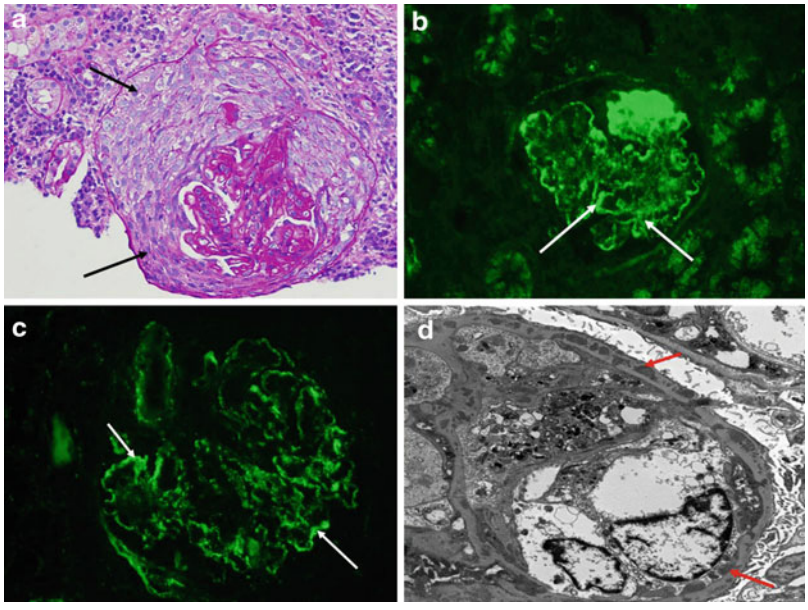
Class IV LN or diffuse proliferative LN is characterized by involvement of more than 50 % of glomeruli, can be global or segmental, and active, chronic, or both. Immunofluorescence and electron microscopy show mesangial and capillary loop immune complex deposits (Image 3). Capillary loop deposits are often large and localize to the subendothelial space. Clinically Class IV LN presents with hematuria, proteinuria that may be in the nephrotic range, and often impaired kidney function.

Membranous LN (Class V)

Pure Class V LN has immune complexes that localize to the mesangium and subepithelial side of the glomerular basement membrane (Image 2). There is no endocapillary hypercellularity. However, Class V LN may occur in combination with Class II, III, or IV LN, and



Lupus Nephritis, Diagnosis and Treatment, Image 2 *Non-proliferative lupus nephritis*: (**a**) Light microscopic image of Class II LN with hematoxylin and eosin stain demonstrating mesangial hypercellularity (*top arrow*) and mesangial matrix expansion (*bottom arrow*). This may also be seen in Class V LN. (**b**) Electron micrograph image of Class V LN with only subepithelial deposits (*arrow*) with classic "humps"



Lupus Nephritis, Diagnosis and Treatment, Image 3 *Proliferative lupus nephritis*: (**a**) Light microscopic image demonstrating cellular crescents (*arrows*). (**b**) Immunofluorescence with IgG staining in mesangium (*right arrow*) and along the basement membrane (*left arrow*). (**c**) Immunofluorescence with C1Q along the basement membrane (*arrows*). (**d**) Electron microscopy with immune complex deposition in the subendothelial space (*bottom arrow*). Intramembranous deposits are also present (*top arrow*)

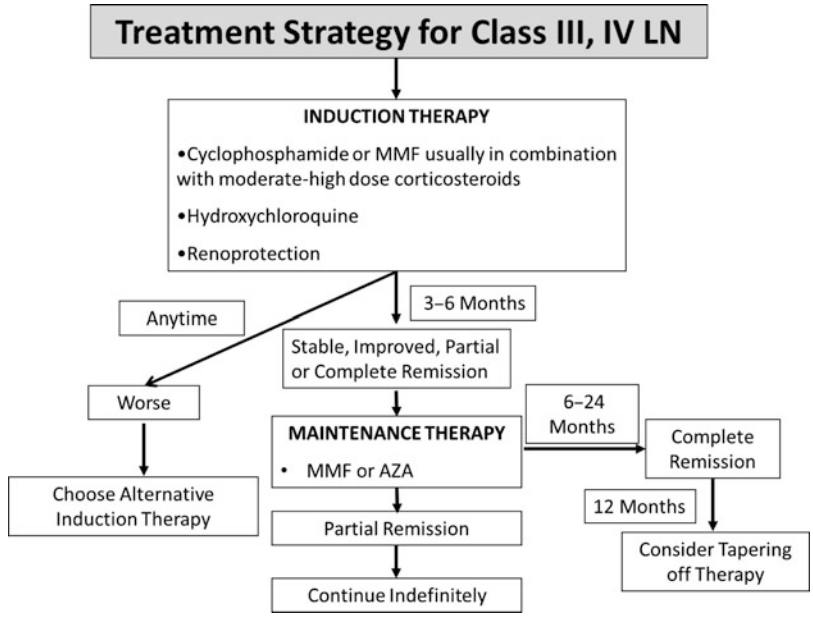
proliferative changes may become the dominant lesions. Clinically Class V demonstrates high-grade proteinuria that is often in nephrotic range, but usually does not present with impaired kidney function, unless associated with one of the proliferative forms of LN.

Advanced Sclerosing LN (Class VI)

Class VI LN is an end-stage category in which over 90 % of glomeruli are globally sclerosed without residual activity. Immunofluorescence and electron microscopy may still show glomerular immune complexes in non-sclerotic glomeruli. These patients typically have advanced chronic kidney disease and ultimately develop end-stage renal disease.

Tubulointerstitial Changes in LN

The presence of tubulointerstitial inflammation is common in LN and typically occurs with



Lupus Nephritis, Diagnosis and Treatment, Fig. 1 Treatment strategy for proliferative lupus nephritis. *MMF* mycophenolate mofetil, *AZA* azathioprine

glomerular disease. The severity of tubulointerstitial involvement is the best marker of renal prognosis (Yu et al. 2010). Interstitial inflammatory cell infiltrates are most common in proliferative forms of LN. If tubular injury progresses, then tubular atrophy and interstitial fibrosis will occur and progressive chronic injury will ensue.

Treatment of Lupus Nephritis

The treatment of LN depends on the type of injury found on kidney biopsy. Classes I and II are typically managed conservatively with emphasis on treating extra-renal symptoms with corticosteroids and/or immunosuppression as indicted. Classes III and IV are often active and aggressive, and are treated with therapies that are designed to quickly "turn off" the disordered immunologic milieu associated with LN. In contrast, if only chronic changes are found on biopsy (e.g., Class VI), the treatment strategy shifts from aggressive immunosuppression to kidney protective measures that will delay progression of kidney disease toward end-stage. These renoprotective therapies are discussed in detail elsewhere in this Encyclopedia, in the entry on "▶ Proteinuric Kidney Diseases: Importance of Blood Pressure Control." Additionally, all LN patients should receive the anti-malarial hydroxychloroquine unless contraindicated. Hydroxychloroquine may protect against vascular thrombosis, chronic kidney disease, renal flares, ESRD, and has a favorable impact on lipid profiles (Tang et al. 2012).

Proliferative LN

Proliferative LN (Class III or IV) is typically an aggressive disease that requires aggressive therapy. The current strategy for treatment is shown in Fig. 1. Treatment is divided into an induction phase and a maintenance phase. During induction, patients are usually treated with one of several cyclophosphamide-based regimens, or a cyclophosphamide-free regimen using mycophenolate mofetil (MMF). Corticosteroids are used with both cyclophosphamide and MMF, most often starting at moderate to high dose, with a taper over several weeks to months. In very severe cases, high-dose intravenous corticosteroids can be used briefly at the beginning of therapy. The duration of induction is typically

6 months for MMF and traditional monthly intravenous pulse cyclophosphamide, 2–4 months for oral cyclophosphamide, or 3 months for low-dose intravenous cyclophosphamide. While induction implies remission, LN does not usually completely remit within 6 months. Thus, treatment for proliferative LN is continued in a maintenance phase, during which the intensity of immunosuppression is reduced to limit toxicity. The two most commonly used medications for maintenance are MMF (in a lower dose than for induction) and azathioprine (AZA).

Cyclophosphamide is a cytotoxic agent that nonspecifically depletes all bone marrow cell lines. MMF and AZA are antimetabolites that prevent B and T cell proliferation. The major complications associated with cyclophosphamide are infertility, future malignancy, and infection. To reduce malignancy risk, the cumulative life time exposure to cyclophosphamide should be <36 g (Faurschou et al. 2008). The most common complications associated with MMF include infection and gastrointestinal complaints such as abdominal pain, nausea, and diarrhea. AZA may also induce bone marrow suppression.

The evolution of the treatment of proliferative LN is beyond the scope of this entry, but demonstrates an evidence-based approach using a foundation of data obtained from experimental models (Austin et al. 1986; Boumpas et al. 1992; Gourley et al. 1996; Contreras et al. 2004; Faurschou et al. 2008; Appel et al. 2009; Grootscholten et al. 2006; Houssiau et al. 2010; Dooley et al. 2011). The goal of treatment was initially to improve mortality of LN, followed by prevention of end-stage kidney disease requiring renal replacement therapy, and more recently, the goal has been to achieve these results with fewer adverse effects. This has prompted the newer low-dose cyclophosphamide and cyclophosphamide-free regimens. The evolution of treatment is not over, and the development and testing of biologic agents that are less toxic and more specifically target immune mediators involved in the pathogenesis of LN are active areas of research.

Membranous LN

Membranous LN (Class V) is a non-proliferative glomerulopathy found in 8–20 % of LN patients (Donadio et al. 1977). It is slowly progressive, but 20 % of patients develop chronic kidney disease, and 8–12 % reach end-stage kidney disease over several years (Sloan et al. 1996). Class V LN with heavy proteinuria therefore requires immunosuppressive treatment, and the general approach to therapy is outlined in Fig. 2 (Austin et al. 2009; Appel et al. 2009).

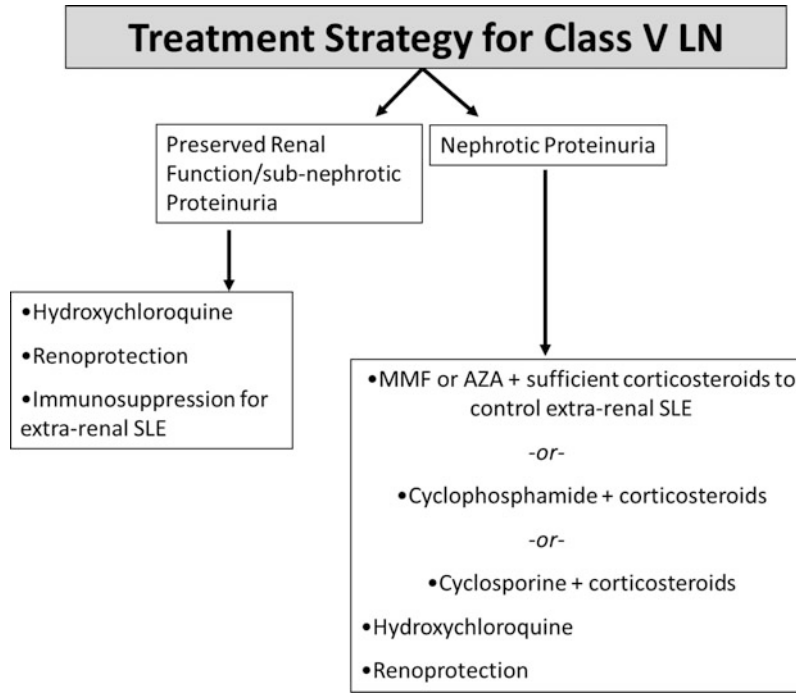
Renal Relapse and Resistant Therapy

LN is a relapsing disease. Almost half of the patients who achieve remission (complete or partial) will experience a renal flare. Flare occurs within a median of 36 months in complete remitters and 18 months in partial remitters (Illei et al. 2002). Renal flare is commonly defined as increase in serum creatinine by ≥25 % and/or an increase in proteinuria above 1 g/day in patients who have completely responded, or a doubling of proteinuria in partial responders. Non-LN causes of an increase in creatinine (medications, infection, dehydration) or proteinuria (excessive salt intake) must be excluded.

There is no consensus definition for resistant LN, but the diagnosis should be considered in patients who have not responded to traditional induction therapy. If one induction regimen fails, an alternative induction agent is typically tried (Fig. 1). If this also fails, a diagnosis of resistant LN can be made. A repeat biopsy may be beneficial to confirm ongoing active disease. Rescue therapies such as rituximab and calcineurin inhibitors have shown some success in small, retrospective trials and may be considered for resistant disease (Vigna-Perez et al. 2006; Cortes-Hernandez et al. 2010).

Anti-phospholipid Syndrome (APS) in SLE

APS is the most common clotting disorder affecting the kidneys in SLE. The incidence of APS in SLE is 30 % and often occurs in



Lupus Nephritis, Diagnosis and Treatment, Fig. 2 Treatment strategy for class V lupus nephritis

conjunction with LN. Lupus anticoagulant is present in 30–50 % of cases and anti-cardiolipin antibodies in 72–95 % of patients, but up to 15 % had neither (Love and Santoro 1990; Daugas et al. 2002). APS describes renal injury due to thrombotic microangiopathy confirmed by renal biopsy. APS is treated with chronic anticoagulation plus hydroxychloroquine. It is non-inflammatory, so immunosuppressive agents are not indicated. Diagnosis of renal APS is imperative as it may lead to CKD/ESRD if not recognized and treated.

Conclusion

LN is a major complication of SLE and is associated with poor outcomes if not recognized early and treated aggressively. Histologic classification is based on ISN/RPS 2003 criteria and proliferative forms of LN require aggressive immunosuppression. Induction therapy with cyclophosphamide or MMF should be followed by maintenance therapy to prevent relapse and progression of CKD. LN relapse rates remain high and current therapies, while effective, are suboptimal, nonspecific, and have significant toxicity. New, more specifically targeted therapies are currently being evaluated to improve response rates and long-term outcomes in LN.

Cross-References

- ▶ Antiphospholipid Syndrome Treatment
- ▶ Antiphospholipid Syndrome, Clinical Manifestations
- ▶ Lupus Nephritis and Novel Therapies, Pathogenesis
- ▶ Novel Targets in Systemic Lupus Erythematosus
- ▶ Proteinuric Kidney Diseases: Importance of Blood Pressure Control
- ▶ Systemic Lupus Erythematosus, Treatment

References

Appel GB, Contreras G, et al. Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. J Am Soc Nephrol. 2009; 20(5):1103–12.

Ardoin S, Birmingham DJ, et al. An approach to validating criteria for proteinuric flare in systemic lupus erythematosus glomerulonephritis. Arthritis Rheum. 2011;63(7):2031–7.

Austin HA. III, Klippel JH, Balow JE, et al. Therapy of Lupus Nephritis: controlled trial of prednisone and cytotoxic drugs. N Engl J Med. 1986;314: 614–9.

Austin 3rd HA, Illei GG, et al. Randomized, controlled trial of prednisone, cyclophosphamide, and cyclosporine in lupus membranous nephropathy. J Am Soc Nephrol. 2009;20(4):901–11.

Birmingham DJ, Rovin BH, et al. Spot urine protein/creatinine ratios are unreliable estimates of 24 h proteinuria in most systemic lupus erythematosus nephritis flares. Kidney Int. 2007;72(7):865–70.

Boumpas DT, Austin H.A. III, et al. Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. Lancet. 1992;340(8822): 741–5.

Cameron JS. Lupus nephritis. J Am Soc Nephrol. 1999;10(2):413–24.

Contreras G, Pardo V, et al. Sequential therapies for proliferative lupus nephritis. N Engl J Med. 2004;350(10):971–80.

Contreras G, Lenz O, et al. Outcomes in African Americans and Hispanics with lupus nephritis. Kidney Int. 2006;69(10):1846–51.

Cortes-Hernandez J, Torres-Salido MT, et al. Long-term outcomes – mycophenolate mofetil treatment for lupus nephritis with addition of tacrolimus for resistant cases. Nephrol Dial Transplant. 2010;25(12):3939–48.

Daugas E, Nochy D, et al. Antiphospholipid syndrome nephropathy in systemic lupus erythematosus. J Am Soc Nephrol. 2002;13(1):42–52.

Donadio Jr JV, Burgess JH, et al. Membranous lupus nephropathy: a clinicopathologic study. Medicine (Baltimore). 1977;56(6):527–36.

Dooley MA, Jayne D, et al. Mycophenolate versus azathioprine as maintenance therapy for lupus nephritis. N Engl J Med. 2011;365(20):1886–95.

Faurschou M, Sorensen IJ, et al. Malignancies in Wegener's granulomatosis: incidence and relation to cyclophosphamide therapy in a cohort of 293 patients. J Rheumatol. 2008;35(1):100–5.

Franco C, Yoo W, et al. Predictors of end stage renal disease in African Americans with lupus nephritis. Bull NYU Hosp Jt Dis. 2010;68(4):251–6.

Gourley MF, Austin HA III, et al. Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis: A randomized controlled trial. Ann Intern Med. 1996;125:549–57.

Grootscholten C, Ligtenberg G, et al. Azathioprine/methylprednisolone versus cyclophosphamide in proliferative lupus nephritis. A randomized controlled trial. Kidney Int. 2006;70(4):732–42.

Houssiau FA, D'Cruz D, et al. Azathioprine versus mycophenolate mofetil for long-term immunosuppression in lupus nephritis: results from the MAINTAIN Nephritis Trial. Ann Rheum Dis. 2010;69(12):2083–9.

Illei GG, Takada K, et al. Renal flares are common in patients with severe proliferative lupus nephritis treated with pulse immunosuppressive therapy: long-term follow up of a cohort of 145 patients participating in randomized controlled studies. Arthritis Rheum. 2002;46(4):995–1002.

Korbet SM, Schwartz MM, et al. Severe lupus nephritis: racial differences in presentation and outcome. J Am Soc Nephrol. 2007;18(1):244–54.

Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med. 1990;112(9):682–98.

Markowitz GS, D'Agati VD. The ISN/RPS 2003 classification of lupus nephritis: an assessment at 3 years. Kidney Int. 2007;71(6):491–5.

Parikh S, Hebert L, Rovin BH. Protecting the kidneys in lupus nephritis. Int J Clin Rheumatol. 2011;6(5):529–46.

Saxena R, Mahajan T, et al. Lupus nephritis: current update. Arthritis Res Ther. 2011;13(5):240.

Seligman VA, Lum RF, et al. Demographic differences in the development of lupus nephritis: a retrospective analysis. Am J Med. 2002;112(9):726–9.

Sloan RP, Schwartz MM, et al. Long-term outcome in systemic lupus erythematosus membranous glomerulonephritis. Lupus Nephritis Collaborative Study Group. J Am Soc Nephrol. 1996;7(2):299–305.

Tang C, Godfrey T, et al. Hydroxychloroquine in lupus: emerging evidence supporting multiple beneficial effects. Intern Med J. 2012

Trendelenburg M, Lopez-Trascasa M, et al. High prevalence of anti-C1q antibodies in biopsy-proven active lupus nephritis. Nephrol Dial Transplant. 2006; 21(11):3115–21.

Venkataseshan VS, Marquet E, et al. Significance of cytoplasmic inclusions in lupus nephritis. Ultrastruct Pathol. 1991;15(1):1–14.

Vigna-Perez M, Hernandez-Castro B, et al. Clinical and immunological effects of Rituximab in patients with lupus nephritis refractory to conventional therapy: a pilot study. Arthritis Res Ther. 2006;8(3):R83.

Woolhandler S, Pels RJ, et al. Dipstick urinalysis screening of asymptomatic adults for urinary tract disorders. I. Hematuria and proteinuria. JAMA. 1989;262(9):1214–9.

Yu F, Wu LH, et al. Tubulointerstitial lesions of patients with lupus nephritis classified by the 2003 International Society of Nephrology and Renal Pathology Society system. Kidney Int. 2010;77(9):820–9.

Lymphocytes in Atherosclerosis

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Synonyms

Atherosclerosis; Atherothrombosis; B lymphocytes; Cardiovascular disease; Coronary artery disease; Lymphocytes; Stroke; T lymphocytes

Definition

Atherosclerosis is a chronic inflammatory disease characterized by the formation of an atherosclerotic plaque or lesion (also called atheroma) in the intima of the artery wall (Crawford et al. 2010). The atheroma is a complex tissue composed of lipids and lipoproteins, infiltrating macrophages and T cells and areas of necrosis and fibrosis. T cells as well as macrophages are found in all stages of disease. Less common B lymphocytes can also be found in the vessel wall and are prevalent in the connective tissue surrounding advanced atheroma, where tertiary lymphoid organs may form. Together, T and B lymphocytes, as the representatives of the adaptive immune system, have been proposed as key modulators of different immunopathogenetic mechanisms on atherosclerosis, e.g., the development of an unstable versus a stable plaque phenotype.

The pathogenic role of these cells has largely been studied in mouse models of human disease. In fact, gene-targeted mice carrying defects in cholesterol metabolism not only develop hypercholesterolemia but also atherosclerosis that is rather similar to the human disease. This has permitted detailed studies of disease mechanisms. Findings made in such models systems obviously require validation in human biobanks, clinical studies, cell culture, and epidemiological investigations (Libby et al. 2011).

Lymphocytes in the Atherosclerotic Lesion

In the advanced human plaque, T cells constitute approximately 10 % of all cells. Around 70 % of these T lymphocytes are $CD4^+$; the remaining ones being largely $CD8^+$ cells, $CD4^+$ lymphocytes can often be found in clusters, suggesting clonal expansion (Jonasson et al. 1986).

Most $CD4^+$ T lymphocytes in the atheroma are of the T helper (Th) 1 subtype and constitute a major source of the pro-atherogenic cytokines interferon gamma (IFNγ) and tumor necrosis factor (TNF), both which have multiple proinflammatory and metabolic actions. However, Th2 cells and/or their signature cytokines have also been identified in the plaque. To a lesser extent, other T lymphocytes are present in the atheroma, including the regulatory T cells (Treg), Th17 cells, and their associated cytokine IL-17, TCR$\gamma\delta^+$ T lymphocytes, and NKT cells (Ketelhuth and Hansson 2011).

Although the immune-mediated reactions in the plaque have been considered to be primarily mediated by macrophages and T lymphocytes, B lymphocytes are also found in human and animal vessel walls, and they accumulate in the periadventitial connective tissue surrounding the atherosclerotic artery. At this site, tertiary lymphoid organs may form, with germinal centers containing B cells in different stages of differentiation to plasma cells, follicular dendritic cells, T cells, macrophages, and conventional dendritic cells (Grabner et al. 2009).

Impact of the Lack of Lymphocytes in Atherosclerosis

The role of lymphocytes in atherosclerosis has been experimentally addressed using immunodeficient mice lacking T and B cells that also

suffered from hypercholesterolemia due to targeted deletions in the *ApoE* or *Ldlr* gene (Daugherty et al. 1997; Song et al. 2001). Such studies showed that lack of adaptive immunity, with an absence of mature T and B cells, led to substantially reduced formation of atherosclerotic lesions. For instance, lesions were reduced by 70 % in *Scid/Scid* x *ApoE*$^{-/-}$ mice (Reardon et al. 2001). Altogether, these data established that although small lesions can develop in the absence of adaptive immunity, T and B lymphocytes play an important role in plaque formation.

Role of Different T Lymphocytes Subsets in Atherosclerosis

The advanced human atherosclerotic plaque contains T cells producing IL-2, IFNγ, and TGF-β (Frostegard et al. 1999). Although TCR (T cell receptor) $\alpha\beta^{+}CD4^{+}$ cells are by far the most frequent ones, $TCR\gamma\delta^{+}$ cells, $CD8^{+}$ T cells, and NKT cells (natural killer T cells) are also detected. Lack of all $CD4^{+}$ T lymphocytes in mice substantially reduces atheroma formation (Zhou et al. 2005). Consequently, adoptive transfer of purified $CD4^{+}$ T lymphocytes from atherosclerotic mice to immunodeficient mice (*ApoE*$^{-/-}$, *scid/scid*) accelerates lesion progression mirrored by increased secretion of IFNγ (Zhou et al. 2000).

The role of Th1 lymphocytes in atheroma formation has been extensively studied. For instance, deletion of the Th1 transcription factor T-bet or deficiency in the Th1 signature cytokine IFNγ leads to reduced plaque size in hypercholesterolemic mice. Abrogation of the action of the anti-inflammatory cytokine TGF-β on T lymphocytes in *ApoE*$^{-/-}$ mice exacerbates Th1 responses, leading to a substantial increase in disease. The role of Th2 lymphocytes in atheroma is not yet very clear. Considered first to be protective against atherosclerosis, the role of Th2 lymphocytes in disease development is controversial. Deficiency in IL-4, the signature Th2 cytokine, leads to less severe disease, implying a pro-atherogenic role of these cells. However, when another Th2 cytokine, IL-5, was targeted in *Ldlr*$^{-/-}$ mice, a protective function was suggested (Hansson and Hermansson 2011; Ketelhuth and Hansson 2011).

Considered to be an effector cell in the context of different autoimmune diseases, i.e., multiple sclerosis, Th17 lymphocytes have been recently evaluated in the context of atherosclerosis. While lack of the Th17 signature cytokine IL-17A or its receptor has been shown to confer protection against atherosclerosis, administration of a recombinant IL-17 limited early lesion development in Ldlr$^{-/-}$ mice. Thus, as for Th2 lymphocytes, further investigations will be necessary to elucidate the role of the Th17 cell subset in atherosclerosis (Ketelhuth and Hansson 2011).

Analysis of T lymphocyte subsets in peripheral blood of patients suffering from atherosclerotic cardiovascular disease showed the expansion of an unusual subset of T lymphocyte, characterized by the lack of CD28 and therefore named $CD4^{+}CD28^{null}$ T lymphocytes. Similarly to Th1 lymphocytes, these cells are characterized by the secretion of high levels of IFNγ and TNF and have been therefore suggested to participate in atheroma development and plaque instability. $CD4^{+}CD28^{null}$ T lymphocytes are expanded in patients with unstable angina and uncommon in patients with stable angina, suggesting also a role in the pathophysiology of atherothrombosis. Unfortunately, $CD4^{+}CD28^{null}$ lymphocytes do not exist in mice limiting research options (Ketelhuth and Hansson 2011).

While in general the response of T lymphocytes is considered pro-atherogenic, the regulatory subsets, i.e., Tregs, are suggested to limit inflammation and plaque formation. Different studies have shown that different subpopulations of Tregs can exert anti-inflammatory functions through the secretion of the cytokines TGF-β and IL-10. In bone marrow transplantation experiments, immunodeficient mice reconstituted with bone marrow deficient in Treg developed significantly increased plaque size (Ait-Oufella et al. 2006). In line with this, defective TGF-β or IL-10 signaling led to

substantially accelerated atherosclerosis (Andersson et al. 2010; Ketelhuth and Hansson 2011).

The role of $CD8^+$ T lymphocytes or CTLs (cytotoxic T lymphocytes) has been in part addressed in the context of atherosclerosis. $CD8^{-/-}$ x $ApoE^{-/-}$ double knockout mice show no major consequences on atheroma development (Elhage et al. 2004). However, the concomitant increase in lesion size and number of $CD8^+$ T lymphocytes in plaques after stimulation with a $CD8^+$ T lymphocyte agonist antibody (Olofsson et al. 2008), anti-CD137, suggests that these cells may be involved in the pathogenesis of this disease.

Role of B Cells in Atherosclerosis

As well as for T lymphocytes, the role of B lymphocytes in atherosclerosis has been studied using different animal models. Reconstitution of irradiated $Ldlr^{-/-}$ using B lymphocyte-deficient bone marrow leads to plaque size increase. Similarly, elimination of a large B lymphocyte population by splenectomy of $ApoE^{-/-}$ mice aggravates atherosclerosis. Interestingly, the latter phenotype can be rescued by adoptive transfer of B lymphocytes (Caligiuri et al. 2002). In this context, B1a lymphocytes have been implicated as the atheroprotective B lymphocyte population. Data suggest that natural IgM secreted by these lymphocytes offers protection by depositing IgM in atherosclerotic lesions, which reduces the necrotic cores of lesions (Kyaw et al. 2011a). Together, these observations suggest a protective role for these cells. However, this concept has been challenged recently. In 2010, two independent studies showed that the depletion of B2 lymphocytes using antibodies to CD20, a glycosylated phosphoprotein expressed on the surface of all B lymphocytes, protected $ApoE^{-/-}$and $Ldlr^{-/-}$ animals against atherosclerosis (Kyaw et al. 2011b).

B lymphocytes can differentiate into plasma cells and secrete antibodies. Antibodies, both IgM and IgG, are also present within atherosclerotic plaques at all stages of lesions development (van Leeuwen et al. 2009). However, if antibodies play a pro- or anti-atherosclerotic role or if they are simply biomarkers of different stages of disease remains unclear.

Proteins Secreted by Lymphocytes Influencing Atherosclerosis

Soluble mediators such as cytokines and antibodies are common soluble factors released by T and B lymphocytes which can modulate atherosclerotic plaque formation, development, and stability. Table 1 illustrates some of the main soluble factors, target cells in the vessel wall, and their potential effects in atherosclerosis.

Antigens Recognized by Lymphocytes in Atherosclerosis

Several autoantigens as well as microbial molecules have been proposed to activate T and B lymphocytes in the atherosclerotic plaque and/or lymphoid organs. LDL is considered to be the major self-antigen implicated in atherogenesis, and it has been suggested that oxidation of the LDL particle breaks immunological tolerance. Human and animal's atherosclerotic plaques present high concentrations of LDL particles. However, T lymphocytes can recognize motifs on native LDL particles, and antibodies are formed to epitopes on native as well as oxidized LDL (Andersson et al. 2010; Ketelhuth and Hansson 2011). Several immunization studies employing LDL preparations have shown atheroprotective effects that have encouraged further research aimed at developing anti-atherosclerotic vaccines. Irrespective of the controversial role of oxidation, recognition of LDL appears to play an important role in the autoimmune process of atherosclerosis.

Also extensively studied, a family of proteins found in mammals and microbes, the heat shock proteins (HSP), has been implicated in atherogenesis. Studies have shown that adaptive immune responses to HSP60 can affect

Lymphocytes in Atherosclerosis, Table 1 Soluble factors released by T and B lymphocytes acting in the plaque

Cytokines	Main lymphocyte sources	Target cells in the vessel wall	Potential functions
IFNγ	Th1 CTL $CD4^+CD28^{null}$ γδ T cells NKT	Macrophages B and T lymphocytes DC EC SMC	↑ adhesion molecules ↑ cytokines and chemokines ↑ MHC-II ↑ co-stimulatory molecules ↑ cytokines and chemokines ↑ MMPs ↓ proliferation ↓ collagen production modulates scavenger receptors
TNFα	Th1 $CD4^+CD28^{null}$ B lymphocytes	Macrophages DCs B and T lymphocytes ECs SMCs	↑ adhesion molecules ↑ cytokines and chemokines ↑ MHC-II ↑ co-stimulatory molecules ↑ MMPs
IL-4	Th2 NKT cells	B and T lymphocytes	B cell proliferation Antibody class switch Plasma cell formation Antibody secretion ↑ Th2-type responses
IL-5	Th2	B and T lymphocytes	B cell differentiation Antibody class switch
IL-17	Th17	Macrophages T lymphocytes SMCs	↑ cytokines and chemokines ↑ MMPs ↑ eicosanoids ↑ ROS ↑ T lymphocyte proliferation
IL-10	Th2 Foxp3+ Tregs Tr1 B lymphocytes	Macrophages B and T lymphocytes DCs ECs SMCs NKT cells	↓ MHC-II ↓ co-stimulatory molecules ↓ MMPs ↓ scavenger receptors ↓ Th1- and Th17-type responses
TGF-β	Foxp3+ Tregs	Macrophages B and T lymphocytes DCs NKT cells EC SMC	↓ adhesion molecules ↓ MHC-II ↓ co-stimulatory molecules ↓ MMPs ↓ scavenger receptors ↑ collagen production ↓ Th1- and Th17-type responses
IgM	B1 lymphocytes	Macrophages DCs	Affect foam cell formation ↑ or ↓ oxLDL uptake Complement activation Killing of bacteria
IgG	B2 lymphocytes	Macrophages DCs	Affect foam cell formation ↑ or ↓ oxLDL uptake Complement activation Anti-inflammatory (FCγRII mediated)

DC dendritic cell, *EC* endothelial cell, *SMC* smooth muscle cell, *NKT* natural killer T cell, *oxLDL* oxidized LDL
For more detailed information about the different T cell subsets involved in atherosclerosis, read the following references: (Song et al. 2001; van Leeuwen et al. 2009; Andersson et al. 2010; Crawford et al. 2010; Hansson and Hermansson 2011; Ketelhuth and Hansson 2011; Kyaw et al. 2011b; Libby et al. 2011).

atherosclerosis (Hansson and Hermansson 2011). Further, other antigens found in the plaque, e.g., beta-2 glycoprotein I (β2GPI), extracellular matrix-derived antigens, and glycation end products (AGE), have been suggested to trigger lymphocyte responses.

Conclusion

Substantial advance has been made in the understanding of the adaptive immune response in atherosclerosis with the discovery of the critical roles of T and B lymphocytes and the complex interactions between these and other immune cell populations.

During the course of plaque formation, the recognition of specific epitopes by lymphocytes in the plaque or in the periphery can critically modulate inflammation in the vessel wall, i.e., pro-atherogenic effects, stimulate (1) endothelial cells to overexpress adhesion molecules, (2) macrophages to release inflammatory cytokines and metalloproteases, and (3) destabilization of the fibrous cup, or anti-atherogenic effects, (1) secretion of anti-inflammatory cytokines, (2) generation of protective antibodies, and (3) thickening of the fibrous cap. Hence, an increasing body of evidence indicates that cellular immunity mediated in special by Th1, Th17, and NKT lymphocytes have pro-atherogenic roles that are counteracted by the protective responses of Tregs and potentially some classes of B cells, e.g., B-1 cells.

Future therapies should aim at reducing the response of pro-atherogenic lymphocytes and favor the expansion of the anti-atherogenic types. Because of the specificity of T and B lymphocytes to their antigens, targeting these cells or their recognizable epitopes may lead to the development of new classes of therapies. Indeed, very promising results arise from the experimental researches. Future work should address whether such responses in human atherosclerosis are similar to that of experimental models and whether similar immunomodulatory strategies for the treatment or prevention of pro-atherosclerotic responses will affect cardiovascular events in humans.

Cross-References

- ▶ Atherosclerosis and Cytokines
- ▶ Macrophages, Oxidative Stress, and Atherosclerosis
- ▶ Systemic Autoimmune Disease and Premature Atherosclerosis
- ▶ Tregs in the Liver

References

Ait-Oufella H, Salomon BL, et al. Natural regulatory T cells control the development of atherosclerosis in mice. Nat Med. 2006;12(2):178–80.

Andersson J, Libby P, et al. Adaptive immunity and atherosclerosis. Clin Immunol. 2010;134(1):33–46.

Caligiuri G, Nicoletti A, et al. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. J Clin Invest. 2002;109(6):745–53.

Crawford MH, DiMarco JP, et al. Cardiology. London: Mosby; 2010.

Daugherty A, Pure E, et al. The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E−/− mice. J Clin Invest. 1997;100(6):1575–80.

Elhage R, Gourdy P, et al. Deleting TCR alpha beta+ or CD4+ T lymphocytes leads to opposite effects on site-specific atherosclerosis in female apolipoprotein E-deficient mice. Am J Pathol. 2004;165(6):2013–8.

Frostegard J, Ulfgren AK, et al. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. Atherosclerosis. 1999;145(1):33–43.

Grabner R, Lotzer K, et al. Lymphotoxin beta receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged *ApoE*−/− mice. J Exp Med. 2009;206(1):233–48.

Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol. 2011;12(3):204–12.

Jonasson L, Holm J, et al. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis. 1986;6(2):131–8.

Ketelhuth DF, Hansson GK. Cellular immunity, low-density lipoprotein and atherosclerosis: break of tolerance in the artery wall. Thromb Haemost. 2011;106(5):779–86.

Kyaw T, Tay C, et al. B1a B lymphocytes are atheroprotective by secreting natural IgM that increases IgM deposits and reduces necrotic cores in atherosclerotic lesions. Circ Res. 2011a;109(8):830–40.

Kyaw T, Tipping P, et al. Current understanding of the role of B cell subsets and intimal and adventitial B cells in atherosclerosis. Curr Opin Lipidol. 2011b;22(5):373–9.

Libby P, Ridker PM, et al. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011;473(7347):317–25.

Olofsson PS, Soderstrom LA, et al. CD137 is expressed in human atherosclerosis and promotes development of plaque inflammation in hypercholesterolemic mice. Circulation. 2008;117(10):1292–301.

Reardon CA, Blachowicz L, et al. Effect of immune deficiency on lipoproteins and atherosclerosis in male apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2001;21(6):1011–6.

Song L, Leung C, et al. Lymphocytes are important in early atherosclerosis. J Clin Invest. 2001;108(2):251–9.

van Leeuwen M, Damoiseaux J, et al. The therapeutic potential of targeting B cells and anti-oxLDL antibodies in atherosclerosis. Autoimmun Rev. 2009;9(1):53–7.

Zhou X, Nicoletti A, et al. Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. Circulation. 2000;102(24):2919–22.

Zhou X, Robertson AK, et al. Lesion development and response to immunization reveal a complex role for CD4 in atherosclerosis. Circ Res. 2005;96(4):427–34.

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Macrophages, Oxidative Stress, and Atherosclerosis

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Synonyms

Atherosclerosis and macrophages

Definition

Vascular inflammation and oxidative stress mediated by macrophages play a key role in the development and progression of atherosclerotic disease. Both of these processes accentuate a proinflammatory milieu which propagates atherosclerosis.

Introduction

Atherosclerotic vascular disease is the leading cause of cardiovascular disease (CVD) and stroke, the two major causes of death in the United States. In response to physiologic stimuli, the vasculature responds dynamically to regulate arterial vascular tone and maintains endothelial integrity and dynamics by producing vasodilators and vasoconstrictors. Risk factors such as diabetes, smoking, hypercholesterolemia, autoimmune disease, and hypertension interfere with this response, promoting macrophage activation, endothelial dysfunction, and atherosclerosis. Recent evidence suggests a central role for vascular inflammation mediated by macrophages and resultant oxidative stress in atherosclerosis and endothelial dysfunction. These studies support the hypothesis that unique oxidants generated in microvasculature and inflammatory cells promote atherosclerosis by lipoprotein oxidation and impairment of macrophage function. Therapies interrupting these oxidative pathways in vascular tissue might help prevent cardiovascular disease.

According to the recent report by the National Vital Statistics, in 2008 over 616,000 people died of heart disease, which translates to roughly 1 in 4 Americans (Minino et al. 2011). CVD remains the leading cause of death for both men and women. In 2008, 405,309 people died from coronary heart disease (CHD). The costs due to health-care services, medications, and loss of productivity for CHD alone have ballooned to an estimated $108.9 billion in 2010 and expected to grow in the future (Heidenreich et al. 2011). Additional manifestations of atherosclerosis include peripheral vascular disease and cerebrovascular events which further increase morbidity, mortality, and economic impact of this process. Therefore, understanding molecular mechanisms of initiation and progression of atherosclerosis has been a prime focus of research of several investigators.

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Some conditions like obesity, diabetes, autoimmune diseases, and kidney disease magnify the burden of atherosclerotic disease by accentuating oxidant stress. In this review, the pivotal role of macrophages and oxidative stress in atherosclerosis and the link between the two processes will be discussed. The potential relationship of lipoproteins and reactive carbonyls to macrophage-derived oxidant-generating pathways and the rationale for therapies aimed at decreasing oxidative stress are emphasized. Identifying specific pathways of reactive oxidant generation in vascular tissue will ultimately lead to design of drugs to interrupt this process and prevent atherosclerotic complications.

Role of Macrophages in Atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by infiltration of lipids and inflammatory cells, such as monocyte-derived macrophages and T-lymphocytes into the artery wall (Ross 1999). Macrophages were the first inflammatory cells to be associated with atherosclerosis. Macrophages produce proinflammatory cytokines, participate in lipid retention and vascular cell remodeling, and express pattern-recognition receptors (PRR), including scavenger receptors (SR) and toll-like receptors (TLRs) that connect the innate and adaptive immune response during atherosclerosis. Macrophages are linked to all stages of atherosclerosis including lesion initiation, propagation of atheroma, and rupture and acute myocardial events (Moore and Tabas 2011). Macrophages can produce reactive oxidants that can damage proteins and lipids and accentuate atherosclerosis (Fig. 1).

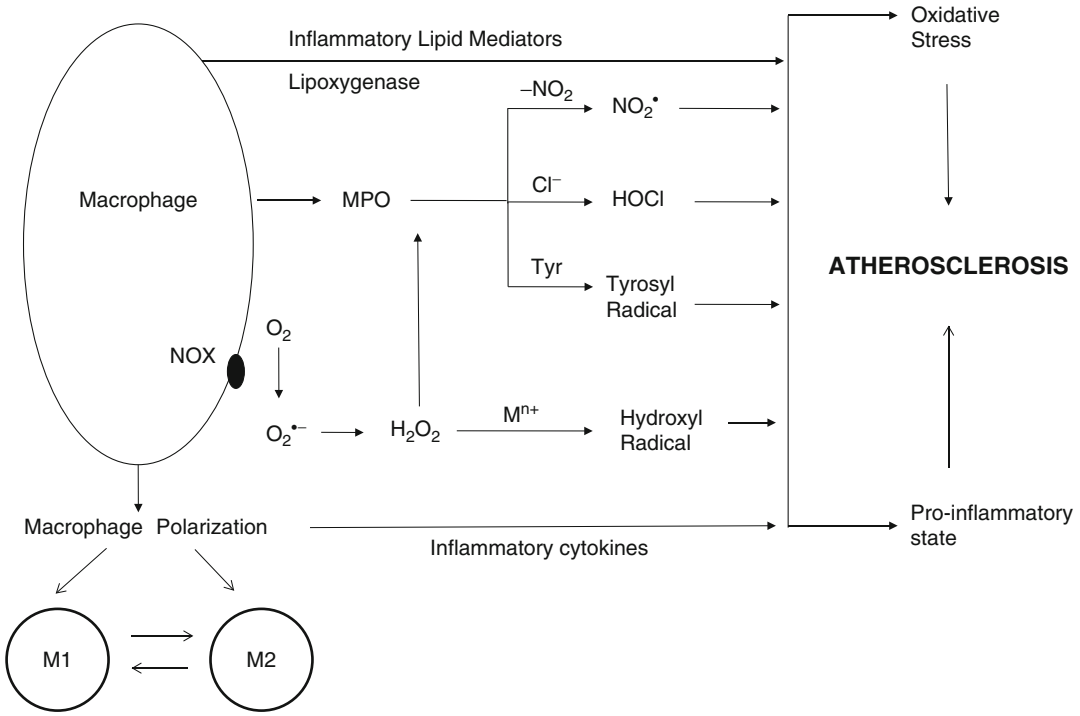
Macrophage Polarization in Atheroma

Mac-1+ cells are the resident macrophages in the arterial wall and express CD11b, CD68, and F4/80. *Classically activated M1* macrophages, driven by *Th1* cytokines (interferon gamma (IFN-γ) and tumor necrosis factor (TNF)) or by lipopolysaccharide (LPS), produce high levels of interleukins 12 and 23 (IL-12 and IL-23) and low levels of interleukin-10 (IL-10) and secrete proinflammatory cytokines. *The alternatively activated protective M2* macrophages are driven by *Th2* cytokines. M1 and M2 macrophages play complimentary but opposing roles during inflammation restraining the process. Indeed, both are present in atherosclerotic lesions, and the balance between the two types determines the degree of vascular inflammation. Flow cytometric analysis of the aortic lesions of low-density lipoprotein (LDL) receptor-deficient mice fed an atherogenic diet for 30 weeks revealed that 39 % of the aortic macrophages express M1 marker CD86, 21 % expressed M2 marker CD206, 45 % expressed Mox marker heme oxygenase 2, and 10 % co-expressed CD86 (Kadl et al. 2010). Upon entry in to the subendothelial space, the macrophages express high levels of scavenger receptor A (SR-A), lipoxygenase 1 (LOX1), chemokine (C-X-C motif) ligand 16 (CXCL16), and CD36.

Endoplasmic reticulum (ER) stress is a key regulator to the macrophage differentiation and cholesterol deposition. When macrophages from diabetics were exposed to oxidized LDL (OxLDL) after either alternative activation into M2 or classic activation into M1, the M2 macrophages formed more foam cells and expressed the scavenger receptors CD36 and SR-A1. ER stress was necessary to generate M2 macrophages through c-Jun N-terminal kinases (JNK) activation and increased peroxisome proliferator-activated receptor (PPAR γ) expression. Absence of SR signaling decreased ER stress and prevented M2 macrophage formation. Furthermore, suppression of ER stress decreased M2 formation and foam cell formation by increasing the high-density lipoprotein (HDL) and Apo lipoprotein A1 (Apo A1)-mediated efflux (Oh et al. 2012).

Oxidized LDL and Atherosclerosis

Although it is well known that elevated levels of LDL greatly increase the risk for atherosclerosis, in vitro studies suggest that LDL by itself is not atherogenic but needs to be modified to initiate atherosclerotic disease. This conclusion led to the "oxidation hypothesis," which proposed that LDL must be oxidatively modified to become



Macrophages, Oxidative Stress, and Atherosclerosis, Fig. 1 *Macrophages-derived oxidants and inflammatory stimuli promote atherosclerosis*. Pathologic conditions result in increased superoxide ($O_2^{\bullet -}$) by macrophages through NADPH oxidase (NOX). $O_2^{\bullet -}$ can dismutate to yield hydrogen peroxide (H_2O_2). H_2O_2 can react with redox-active metal ions (M^{n+}) to form hydroxyl radical or with phagocyte-derived myeloperoxidase (MPO) to nitrate (nitrogen dioxide; $NO_2^{\bullet}$), chlorinate (hypochlorous acid; HOCl), or cross-link (tyrosyl radical) proteins. Macrophages can also promote a proinflammatory milieu by generation of a multitude of cytokines which can overwhelm endogenous anti-inflammatory antioxidant defenses resulting in atherosclerosis

atherogenic. Many lines of evidence support this hypothesis. OxLDL is taken up by SR-A and CD 36 of macrophages, which then become lipid-laden foam cells, the pathologic hallmark of early atherosclerotic lesions. OxLDL has been isolated from human and animal atherosclerotic tissue, and immunohistochemical studies have detected oxidized lipids in atherosclerotic lesions. All major cell types involved in atherosclerosis – smooth muscle cells, endothelial cells, and macrophages – produce reactive oxidants that can oxidize LDL in vitro. Moreover, OxLDL attracts mononuclear cells and stimulates the production of monocyte chemoattractant protein-1 (MCP-1) and other inflammatory cytokines such as macrophage colony-stimulating factor (M-CSF), leading to the conversion of fatty streaks to more advanced complex lesions as smooth muscle cells migrate from the media into the subendothelial space. OxLDL may also stimulate smooth cells to synthesize extracellular matrix and activate a signaling cascade by interacting with the lectin-like OxLDL receptor. Finally, several structurally unrelated lipid-soluble antioxidants that inhibit LDL oxidation in vitro also inhibit atherosclerosis in hypercholesterolemic animals (reviewed in Vivekanadan-Giri et al. 2008).

There is some controversy about the function of scavenger receptors in atherogenesis. Early studies showed that SR plays a pro-atherogenic role in atherosclerosis. Crossing $CD36^{-/-}$ null mice on apolipoprotein E (ApoE)-deficient background showed protection from atherosclerosis (Febbraio et al. 2000). In a separate study, $ApoE^{-/-}$ mice lacking SR-A or CD36 showed

increased aortic sinus lesion areas with abundant foam cells, suggesting alternative lipid uptake mechanisms and a possible athero-protective role for SR-A and CD36 (Moore et al. 2005). $ApoE^{-/-}$ mice with a combined deficiency of CD36 and SR-A I/II showed no further reduction of atherosclerosis compared with $Cd36^{-/-}ApoE^{-/-}$ mice (Kuchibhotla et al. 2008).

High-Density Lipoproteins and Reverse Cholesterol Transport

HDL, a lipid-protein complex, has long been recognized for its inverse association with CVD, largely attributed to its ability to remove excess cholesterol from arterial wall macrophages, a process known as reverse cholesterol transport (RCT; Tall et al. 2008). Once internalized, free cholesterol trafficking to the cell membrane may play a role in lesion regression, and deficiency in this pathway in certain macrophages might be the reason for lesion progression. Once at the membrane, the ABCA1 (ATP-binding cassette protein A1)- and ABCG1 (ATP-binding cassette G1)-mediated transfer to Apo A1 and HDL respectively occurs (Tall et al. 2008). Passive diffusion occurs as well.

Along with its important role in mediating RCT, HDL has multiple endothelial actions that also afford cardiovascular protection, including antioxidative, anti-inflammatory, antiapoptotic, and antithrombotic activities. Further, HDL activates endothelial nitric oxide (NO) synthase (Besler et al. 2011), enhances endothelial progenitor cell-mediated endothelium repair, and stimulates endothelial cell proliferation and migration. Additionally, increasing the levels of HDL by drugs, gene therapy, or direct infusion has been shown to improve outcomes in animal models and in patients with CVD.

Role of Macrophages in Thinning of Fibrous Cap/Plaque Rupture

The older and mature plaques rupture and ulcerate at the shoulder regions enriched with macrophages and macrophage-derived foam cells. Macrophages trigger smooth muscle cell apoptosis by activating apoptotic pathways and by secreting TNF-α and NO (Boyle et al. 2003). Macrophages also decrease transforming growth factor (TGF) β production causing decrease in collagen production (Fadok et al. 1998). With prolonged ER stress, the unfolded protein response (UPR) effector C/EBP homologous protein (CHOP) can trigger UPR. Macrophages secrete metalloproteinases (MMP) which can lead to thinning of the fibrous cap due to their protease activity and may predispose to the unstable plaque and rupture (Tabas 2010).

Macrophages produce tissue plasminogen activator (TPA) and plasminogen activator inhibitor type 1(PAI-1) for control of local fibrinolysis. PAI-1 and alpha 2-antiplasmin are elevated in atherosclerotic plaques while TPA is low, which may favor thrombosis and predispose to acute MI (Schwartz et al. 1988).

Diabetes Promotes Inflammatory Macrophage Phenotype

Recent evidence points out to direct effects of diabetes on atherosclerotic lesion cells, such as macrophages, which play an additional important role in accounting for increased atherosclerotic risk in diabetes. Thus, the increased macrophage expression of inflammatory mediators associated with diabetes can be mimicked by elevated glucose concentrations in vitro (Wen et al. 2006). In addition, fatty acids exert inflammatory effects in macrophages, which could contribute to inflammation in the setting of diabetes-accelerated atherosclerosis and possibly other complications (Hummasti and Hotamisligil 2010). After entering the cell, fatty acids are thio-esterified into their acyl-CoA derivatives catalyzed by long-chain acyl-CoA synthetases (ACSLs). Kanter et al. (2012) demonstrated that monocytes from humans and mice with type 1 diabetes also exhibit increased ACSL1. Furthermore, myeloid-selective deletion of ACSL1 protected monocytes and macrophages from the inflammatory effects of diabetes. Strikingly, myeloid-selective deletion of ACSL1 also prevents accelerated atherosclerosis in diabetic

mice without affecting lesions in nondiabetic mice (Kanter et al. 2012). These observations indicate that ACSL1 plays a critical role by promoting the inflammatory phenotype of macrophages associated with diabetes.

Macrophage Sterol Regulation and Atherosclerosis

Sterol-regulated transcriptional factors liver X receptors (LXR α and β) appear to be anti-atherogenic as mice lacking LXR's have advanced atherosclerosis and LXR agonists decrease atherosclerosis. Transplanting aortic arches from atherosclerotic ApoE-deficient mice with LXR α and LXR β deficiency into wild-type mice resulted in marked regression of atherosclerotic lesions (Feig et al. 2010). The salutary effects of LXR's in part are thought to be due to upregulation of RCT due to increased ABCA1 and ABCG1 activity in macrophages. Activation of PPAR γ can inhibit foam cell formation with and without help from the ABCA1 pathways. PPAR γ activation reduces cholesterol esterification and induces ABCG1 expression. Becker et al. showed that the peritoneal macrophages from chow- versus high-fat-fed LDL receptor-deficient mice differentially expressed cytoskeleton, lipid trafficking, and lipid-binding proteins utilizing mass spectrometry (MS)-based proteomic techniques (Becker et al. 2010). Their analysis revealed a sterol-responsive network that is highly enriched in proteins with known physical interactions and established roles in vesicular transport associated with atherosclerotic phenotypes in mice. Pharmacologic intervention with a statin or rosiglitazone and use of mice deficient in LDL receptor or ApoE implicated the network in atherosclerosis. Biochemical fractionation revealed that most of the sterol-responsive proteins resided in microvesicles, providing a physical basis for the network's functional and biochemical properties. These observations identify a highly integrated network of macrophage proteins whose expression is influenced by environmental, genetic, and pharmacological factors implicated in atherogenesis.

Role of Oxidative Stress in Atherosclerosis

Although oxidative stress has a well-established role in atherosclerosis, its origins and magnitude remain poorly understood. Moreover, it is not known whether oxidative stress is a primary event that occurs early in the disease or whether it represents a secondary phenomenon that merely reflects end-stage tissue damage. This distinction has important clinical relevance. If oxidative stress simply reflects tissue damage, interventions that reduce it may fail to affect the disease process. If oxidative stress promotes tissue injury, therapies that interrupt oxidative pathways early in the disease may prevent complications, and those that act later may slow disease progression.

Pathways for Generating Oxidants

Many pathways oxidize proteins in vitro. However, the specific pathways that promote oxidative stress in atherosclerosis have not been conclusively identified. One reason is that oxidizing intermediates are difficult to detect in vivo because they are short lived and generated at low levels.

Macrophage-Derived Oxidants

(a) *The NADPH pathway*. The major pathway through which macrophages and other phagocytic cells of the innate immune system generate oxidants begins with the cell membrane-bound NADPH oxidase (NOX), which produces superoxide. Superoxide dismutates into hydrogen peroxide which can be used as a substrate for propagating oxidative reactions by free metal ions (hydroxyl radical) or used as substrate by myeloperoxidase (MPO). Superoxide can also react with NO to form the potent oxidant peroxynitrite. Several NOX isoforms are present in the endothelium, and smooth muscle cells are selectively upregulated by pathologic stimuli, which can augment macrophage-derived oxidants. Potential factors

include angiotensin II, endothelin-1, hypercholesterolemia, shear stress, nonesterified fatty acids, hyperglycemia, and growth factors. Angiotensin II may represent a pathophysiologically relevant pathway for stimulating the production of reactive intermediates by artery wall cells because inhibitors of this pathway lower the risk for cardiovascular events. In humans, NOX activity correlates inversely with endothelial function, even after other major risk factors for atherosclerosis, including diabetes and hypercholesterolemia, are taken into account (Guzik et al. 2006).

(b) *The MPO pathway*. The peroxide generated by NOX can be used by another phagocyte enzyme, MPO, to convert chloride ion to hypochlorous acid (HOCl). Oxidation of NO with oxygen yields nitrite (NO_2^-), which MPO converts to nitrogen dioxide radical ($NO_2^{\bullet}$), a potent nitrating intermediate (Gaut et al. 2002). Reactive nitrogen species, including peroxynitrite and $NO_2^{\bullet}$, might contribute to the inflammatory process by nitrating lipoproteins and other biomolecules. Hyperglycemia can activate protein kinase C (PKC) (Ishii et al. 1998), which leads to phagocyte activation, secretion of MPO, and oxidant generation. Nonesterified fatty acids (NEFA) that commonly are overabundant in diabetes can also activate phagocytes in vitro. These changes might enhance the production of superoxide and hydrogen peroxide, which myeloperoxidase converts into more potent cytotoxic oxidants, such as HOCl and $NO_2^{\bullet}$.

MPO is a major source of reactive oxidants in the human vasculature and has been widely implicated in the development of human atherosclerotic lesions. In large epidemiological studies, the level of plasma MPO is a strong independent predictor of CVD (Brennan et al. 2003). Further, MPO has been localized to atherosclerotic plaques, and oxidants produced by MPO activate protease cascades and plaque rupture (Fu et al. 2001). Lipoproteins that have been modified by MPO and HOCl have also been detected in human atherosclerotic lesions (Vivekanadan-Giri et al. 2008). For example, MPO has been linked to LDL oxidation, which triggers the formation of lipid-laden foam cells. Oxidants produced by MPO also activate protease cascades that weaken atherosclerotic plaques, increasing the likelihood of rupture (Fu et al. 2001). Moreover by consuming the NO that relaxes blood vessel walls, MPO may cause vasoconstriction and endothelial dysfunction and reduce anti-inflammatory function (Baldus et al. 2006). MPO is also a source of reactive nitrogen species because it generates nitrotyrosine in vitro and in vivo (Gaut et al. 2002). Expression of human MPO in macrophages accelerated atherosclerosis in mice (McMillen et al. 2005), strongly supporting the hypothesis that the enzyme's ability to produce reactive intermediates promotes vascular disease. Consistent with this potential pro-atherogenic role, certain MPO polymorphisms associate with cardiovascular risk. Vita and colleagues (Vita et al. 2004) examined the relationship between serum MPO levels and endothelial function (measured with flow-mediated and nitroglycerin-mediated dilation of the brachial artery) in 298 subjects with or without CVD. Correlates of vasodilator function included established CVD risk factors, such as age and HDL level, as well as serum MPO level. When the data were stratified into quartiles, MPO levels predicted endothelial dysfunction even after multivariate adjustment.

MPO binds to glycosaminoglycans in vessel walls, where it has been proposed to oxidize endothelium-derived NO and impair endothelial function. Baldus et al. (2006) demonstrated that heparin mobilizes MPO from vascular compartments in humans with and without CVD. Thus, administration of heparin increased plasma levels of MPO. Heparin treatment also improved endothelial NO bioavailability, as monitored by flow-mediated dilation and acetylcholine-induced changes in forearm blood flow. Improved endothelial function correlated with the

extent of heparin-induced MPO release. These observations suggest that MPO represents a mechanism for heparin's anti-inflammatory effects and that it affects vascular NO bioavailability.

(c) *The reactive nitrogen pathway*. Another pathway for generating oxidants involves nitric oxide (NO). NO is produced during inflammation by macrophages, which are early components of atherosclerotic lesions from inducible nitric oxide synthase (iNOS). NO reacts with superoxide ($O_2^{\bullet -}$) to generate peroxynitrite, a potent oxidant that converts tyrosine residues to 3-nitrotyrosine. Thus, 3-nitrotyrosine is a marker for the reactive nitrogen pathway. It has been detected in low-density and high-density lipoproteins (LDL and HDL) isolated from human diabetic atherosclerotic lesions (Pennathur et al. 2004), and plasma nitrotyrosine levels are elevated in patients with CHD (Shishehbor et al. 2003). Because acute hyperglycemia promotes vasodilation in humans, glucose might directly or indirectly enhance NO release and oxidant generation.

Other Oxidant Pathways That Promote Oxidative Stress in the Vascular Wall

There are several sources of non-macrophage-derived oxidants in the artery wall that can propagate oxidative stress in the vascular wall. These include the glycoxidation pathway, which involves glucose-derived oxidants which generate advanced glycosylation end products (AGEs), the mitochondrial electron transport chain, uncoupled endothelial nitric oxide synthase, xanthine oxidase, and non-phagocytic NOX enzymes. These pathways can accentuate oxidative damage in a pro-atherogenic milieu.

Experimental Studies Identifying Pathways of Oxidation in Atherosclerosis

Mass spectrometry (MS) is a powerful approach for detecting biomarkers of oxidative stress in vivo. Antibody-based assays and dihydroethidium fluorescence have been extensively used to study oxidation-specific epitopes and oxidant production in atherosclerosis. These techniques are highly sensitive, and the ability of immunochemical studies to provide anatomical data can localize oxidative events. However, they are nonspecific and, at best, only semiquantitative. In contrast, MS offers a powerful set of analytical tools for quantifying and identifying biomolecules. Isotope dilution gas or liquid chromatography (GC and LC)/MS is a highly sensitive and specific method that is used to quantify oxidation of specific amino acid markers (reviewed in Vivekanandan-Giri et al. 2011). Biomolecules such as oxidized amino acids derived from plasma or tissue are separated by GC or LC and ionized. The *mass-to-charge* ratios of ions derived by fragmenting the ionized, parent compound are determined by MS. Such a spectrum can unequivocally identify a target biomolecule because each compound has a unique fragmentation pattern. The analyte is quantified by adding a stable, isotopically labeled internal standard, which is identical to the target analyte except for the heavy isotope. With certain ionization processes, such as electron capture negative-ion chemical ionization, it is possible to detect and quantify sub-femtomole levels of biomolecules.

Oxidized Amino Acids Serve as Molecular Fingerprints for Specific Oxidation Pathways

To understand the molecular mechanisms that promote oxidative stress in vivo, a chemical approach to define the patterns of oxidation products that are formed by well- characterized oxidant-generating systems in vitro has been employed. Similar patterns in tissue and plasma were identified. Focus was on proteins because aromatic amino acids constituting proteins retain the initial footprint of the reactive intermediate that initiates oxidative damage. In contrast, lipid peroxidation products readily undergo subsequent chain-propagating reactions, which mask the products formed early in the pathway. Moreover, many different oxidizing intermediates give the same spectrum of oxidized lipids, making it difficult to identify specific pathways that trigger lipid oxidation.

Oxidizing intermediates are difficult to detect in vivo because they are short lived and generated

at low levels. To sidestep this problem, acid-stable products of protein oxidation were identified and monitored, both in vitro and in vivo (Bhattacharjee et al. 2001; Pennathur et al. 2001, 2004, 2005). The overall approach is to use isotope dilution MS to accurately identify oxidized amino acids isolated from tissue proteins. These markers, which include *ortho*-tyrosine, *meta*-tyrosine, dityrosine, 3-nitrotyrosine, and 3-chlorotyrosine indicate which biochemical pathway has damaged a protein (reviewed in Vivekanadan-Giri et al. 2008, 2011).

MS Quantitation of Oxidized Amino Acids in Aortic Proteins of Diabetic *Cynomolgus* Monkeys Reveals Localized Oxidative Damage by Hydroxyl Radical in the Artery Wall

To investigate the potential role of localized oxidative stress in diabetic macrovascular disease, *cynomolgus* monkeys that had been hyperglycemic for 6 months due to streptozotocin-induced diabetes were used (Pennathur et al. 2001). Samples from seven controls and eight diabetic *cynomolgus* monkeys were analyzed, sampling three different areas from the thoracic aorta of each animal. Compared with the control samples, the aortic wall proteins from the diabetic animals showed a significant (40 %) increase in *ortho*-tyrosine and a similar (60 %) increase in *meta*-tyrosine. This pattern of oxidized amino acids suggests that a hydroxyl radical-like oxidant promotes aortic damage in this animal model of diabetic vascular disease. To determine whether glucose promotes protein oxidation in vivo, we assessed the relationship between level of glycemic control (measured as serum glycated hemoglobin) and levels of amino acid oxidation products in aortic tissue in control and diabetic *cynomolgus* monkeys. Linear regression analysis demonstrated a strong correlation between levels of both *ortho*-tyrosine and *meta*-tyrosine and glycated hemoglobin ($r^2 = 0.9$ and 0.8, respectively; both $p < 0.001$). Levels of *o*,*o*′-dityrosine and glycated hemoglobin correlated less strongly ($r^2 = 0.3$; $p = 0.07$). In contrast, there was no correlation between levels of 3-nitrotyrosine and glycated hemoglobin. These observations support the hypothesis that glucose promotes the formation of *ortho*-tyrosine and *meta*-tyrosine in the artery wall and suggest that both glucose and other pathways contribute to the generation of *o*,*o*′-dityrosine (Pennathur et al. 2001).

Plasma and Urinary Levels of Oxidized Amino Acids Are Potential Markers for Assessing Oxidative Stress In Vivo

There is increasing evidence that oxidized amino acids in plasma and urine can serve as markers for noninvasive assessment of oxidative stress in vivo. Plasma and urinary levels of these markers are likely to be proportional to the rate of generation and thus can serve as indices of chronic oxidative stress in vivo (Bhattacharjee et al. 2001; Bergt et al. 2004). These observations may be relevant to human pathophysiology. For example, a case–control study demonstrated that systemic levels of protein-bound nitrotyrosine were significantly higher in patients with CVD than in controls with healthy arteries. Moreover, statin therapy lowered levels of oxidation markers in plasma, raising the possibility that these drugs can potentially be antioxidants. Therefore, these markers might serve not only to assess oxidative stress but also to monitor the efficacy of therapy.

HDL Oxidation and Altered HDL Proteome in Atherosclerosis

Oxidized amino acids in plasma lipoproteins are markers of lipoprotein oxidation which are implicated in atherogenesis. Preliminary studies demonstrated that HDL, but not LDL, isolated from plasma of subjects with established CVD contained high levels of 3-chlorotyrosine, a highly specific marker for the myeloperoxidase pathway. The level of 3-chlorotyrosine was 13-fold higher in HDL isolated from plasma of subjects with established CVD than in HDL from plasma of healthy subjects (Bergt et al. 2004). Levels of 3-nitrotyrosine were twice as high in

HDL from plasma of patients with established CVD (Pennathur et al. 2004). Circulating HDL from patients with known CVD has impaired reverse cholesterol transport (Bergt et al. 2004; Pennathur et al. 2004; Shao et al. 2012). HDL isolated from atherosclerotic plaques is bound to MPO and enriched in MPO-derived oxidation products, supporting an important role of MPO in the development of dysfunctional HDL. Proteomic approaches were applied to the composition of HDL isolated from healthy subjects and subjects with CVD. Multiple complement regulatory proteins and a diverse array of distinct serpins with serine-type endopeptidase inhibitor activity were identified. Many acute-phase response proteins were also detected, supporting the proposal that HDL is of central importance in inflammation. MS and biochemical analyses demonstrated that HDL3 from subjects with CVD was selectively enriched in ApoE, raising the possibility that HDL carries a unique cargo of proteins in humans with clinically significant CVD. HDL protein cargo plays a role in regulating the complement system and protecting tissue from proteolysis and contributes to its anti-inflammatory and anti-atherogenic properties (Vaisar et al. 2007). These observations raise the exciting possibility that oxidized HDL and HDL proteome might be a novel marker for clinically significant CVD. Furthermore, it highlights the importance of MPO as a mechanistic cause of atherosclerosis.

Lipoxidation and Atherosclerosis

Lipoxygenases

The LOX enzymes are present in macrophages and are implicated in human atherosclerosis. They are named for the numbered carbon where they oxygenate their polyunsaturated fatty acid (PUFA) substrates (e.g., 12-LOX and 5-LOX). The human LOX enzymes include 5-LOX (which produces leucotrienes), 12-LOX (with platelet-type and leukocyte-type forms), and 15-LOX (which is further separated into the reticulocyte or leukocyte-type, 15-LOX-1). The human leukocyte-type 12-LOX and the human reticulocyte-type 15-LOX-1 can form similar products from common substrates and are often referred to in the literature as 12/15-LOXs (Dobrian et al. 2011).

In humans, 12/15-LOXs act upon arachidonic acid to form a number of important lipid mediators including 12- and 15-hydroperoxyeicosatetraenoic acids (HPETEs), 12- and 15-hydroxyeicosatetraenoic acids (HETEs), and hydroxyoctadecadienoic acids (HODEs) from linoleic acid. These lipid products in addition to oxidizing LDL can have a myriad of proinflammatory functions that can propagate atherosclerosis. While the pro-atherogenic role of the 5-LOX pathway is generally better established in animal models and human studies, the role of the 12- and 15-LOX pathways is not yet clear. Indeed, some 15-LOX metabolites are known to be anti-inflammatory and promote a more anti-inflammatory macrophage phenotype in the vessel wall.

Hyperlipidemia in Concert with Hyperglycemia Stimulates the Proliferation of Macrophages in Atherosclerotic Lesions: Potential Role of Glucose-Oxidized LDL

Macrophage proliferation has been implicated in the progression of atherosclerosis. Recent studies have investigated the effects of hyperglycemia and hyperlipidemia on macrophage proliferation in murine atherosclerotic lesions and isolated primary macrophages (Lamharzi et al. 2004). Glucose promoted lipid and protein oxidation of LDL in vitro (Pennathur et al. 2005). Oxidation of LDL with glucose resulted in a selective increase in protein-bound *ortho*-tyrosine and *meta*-tyrosine. Moreover, glucose-oxidized LDL – but not elevated levels of glucose alone – stimulated proliferation of isolated macrophages. These observations may be pertinent to diabetic vascular disease because macrophage proliferation in atherosclerotic lesions was observed in LDL receptor-deficient mice that were both hypercholesterolemic and hyperglycemic but not in mice that were only hyperglycemic (Lamharzi et al. 2004).

Conclusion and Perspectives

Many lines of evidence implicate macrophages and oxidative stress in atherosclerosis. Macrophages play a critical determinant in initiation and progression of atherosclerosis, and its effect, is in part mediated by oxidative stress. Measuring levels of specific oxidized biomolecules by MS show promise for evaluating the role of oxidative stress in the pathogenesis of vascular disease and determine therapeutic efficacy of agents that promote diminished vascular inflammation. Our observations also suggest that chlorinated and/or nitrated HDL might serve as a marker – and perhaps a mechanism – of active cardiovascular disease in humans. If oxidation of HDL by MPO converts the cardioprotective lipoprotein into a dysfunctional form, the enzyme might be a suitable therapeutic target for preventing vascular disease. Additionally agents that improve HDL function and ability to perform RCT would potentially have a major therapeutic role. These include LXR agonists, creating oxidant resistant forms of HDL, peptide, or small molecule HDL mimetics. Additionally, agents that would alter macrophage phenotype to anti-inflammatory state would also be beneficial in preventing CVD.

Cross-References

► Dendritic Cells in Atherosclerosis

References

Baldus S, Rudolph V, et al. Heparins increase endothelial nitric oxide bioavailability by liberating vessel-immobilized myeloperoxidase. Circulation. 2006;113(15):1871–8.

Becker L, Gharib SA, et al. A macrophage sterol-responsive network linked to atherogenesis. Cell Metab. 2010;11(2):125–35.

Bergt C, Pennathur S, et al. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. Proc Natl Acad Sci USA. 2004;101(35):13032–7.

Besler C, Heinrich K, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest. 2011;121(7):2693–708.

Bhattacharjee S, Pennathur S, et al. NADPH oxidase of neutrophils elevates *o*,*o*′-dityrosine cross-links in proteins and urine during inflammation. Arch Biochem Biophys. 2001;395(1):69–77.

Boyle JJ, Weissberg PL, et al. Tumor necrosis factor-alpha promotes macrophage-induced vascular smooth muscle cell apoptosis by direct and autocrine mechanisms. Arterioscler Thromb Vasc Biol. 2003;23(9):1553–8.

Brennan ML, Penn MS, et al. Prognostic value of myeloperoxidase in patients with chest pain. N Engl J Med. 2003;349(17):1595–604.

Dobrian AD, Lieb DC, et al. Functional and pathological roles of the 12- and 15-lipoxygenases. Prog Lipid Res. 2011;50(1):115–31.

Fadok VA, Bratton DL, et al. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest. 1998;101(4):890–8.

Febbraio M, Podrez EA, et al. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. J Clin Invest. 2000;105(8):1049–56.

Feig JE, Pineda-Torra I, et al. LXR promotes the maximal egress of monocyte-derived cells from mouse aortic plaques during atherosclerosis regression. J Clin Invest. 2010;120(12):4415–24.

Fu X, Kassim SY, et al. Hypochlorous acid oxygenates the cysteine switch domain of pro-matrilysin (MMP-7). A mechanism for matrix metalloproteinase activation and atherosclerotic plaque rupture by myeloperoxidase. J Biol Chem. 2001;276(44):41279–87.

Gaut JP, Byun J, et al. Myeloperoxidase produces nitrating oxidants in vivo. J Clin Invest. 2002;109(10):1311–9.

Guzik TJ, Sadowski J, et al. Coronary artery superoxide production and NOX isoform expression in human coronary artery disease. Arterioscler Thromb Vasc Biol. 2006;26(2):333–9.

Heidenreich PA, Trogdon JG, et al. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation. 2011;123(8):933–44.

Hummasti S, Hotamisligil GS. Endoplasmic reticulum stress and inflammation in obesity and diabetes. Circ Res. 2010;107(5):579–91.

Ishii H, Daisuke K, et al. Protein kinase C activation and its role in the development of vascular complications in diabetes mellitus. J Mol Med. 1998;76:21–31.

Kadl A, Meher AK, et al. Identification of a novel macrophage phenotype that develops in response to atherogenic phospholipids via Nrf2. Circ Res. 2010;107(6):737–46.

Kanter JE, Kramer F, et al. Diabetes promotes an inflammatory macrophage phenotype and atherosclerosis through acyl-CoA synthetase 1. Proc Natl Acad Sci USA. 2012;109(12):4353–4.

Kuchibhotla S, Vanegas D, et al. Absence of CD36 protects against atherosclerosis in ApoE knock-out mice with no

additional protection provided by absence of scavenger receptor A I/II. Cardiovasc Res. 2008;78(1):185–96.
Lamharzi N, Renard CB, et al. Hyperlipidemia in concert with hyperglycemia stimulates the proliferation of macrophages in atherosclerotic lesions: potential role of glucose-oxidized LDL. Diabetes. 2004;53 (12):3217–25.
McMillen TS, Heinecke JW, et al. Expression of human myeloperoxidase by macrophages promotes atherosclerosis in mice. Circulation. 2005;111(21):2798–804.
Minino AM, Murphy SL, et al. Deaths: final data for 2008. Natl Vital Stat Rep. 2011;59(10).
Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. Cell. 2011;145(3):341–55.
Moore KJ, Kunjathoor VV, et al. Loss of receptor-mediated lipid uptake via scavenger receptor A or CD36 pathways does not ameliorate atherosclerosis in hyperlipidemic mice. J Clin Invest. 2005;115(8):2192–201.
Oh J, Riek AE, et al. Endoplasmic reticulum stress controls M2 macrophage differentiation and foam cell formation. J Biol Chem. 2012;287(15): 11629–41.
Pennathur S, Wagner JD, et al. A hydroxyl radical-like species oxidizes cynomolgus monkey artery wall proteins in early diabetic vascular disease. J Clin Invest. 2001;107(7):853–60.
Pennathur S, Bergt C, et al. Human atherosclerotic intima and blood of patients with established coronary artery disease contain high density lipoprotein damaged by reactive nitrogen species. J Biol Chem. 2004;279(41):42977–83.
Pennathur S, Ido Y, et al. Reactive carbonyls and polyunsaturated fatty acids produce a hydroxyl radical-like species: a potential pathway for oxidative damage of retinal proteins in diabetes. J Biol Chem. 2005;280(24):22706–14.
Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med. 1999;340(2):115–26.
Schwartz CJ, Valente AJ, et al. Thrombosis and the development of atherosclerosis: Rokitansky revisited. Semin Thromb Hemost. 1988;14(2):189–95.
Shao B, Pennathur S, et al. Myeloperoxidase targets apolipoprotein A-I, the major high density lipoprotein protein, for site-specific oxidation in human atherosclerotic lesions. J Biol Chem. 2012;287(9):6375–86.
Shishehbor MH, Brennan ML, et al. Statins promote potent systemic antioxidant effects through specific inflammatory pathways. Circulation. 2003;108(4): 426–31.
Tabas I. The role of endoplasmic reticulum stress in the progression of atherosclerosis. Circ Res. 2010;107(7): 839–50.
Tall AR, Yvan-Charvet L, et al. HDL, ABC transporters, and cholesterol efflux: implications for the treatment of atherosclerosis. Cell Metab. 2008;7(5):365–75.
Vaisar T, Pennathur S, et al. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J Clin Invest. 2007;117(3):746–56.
Vita JA, Brennan ML, et al. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation. 2004;110(9):1134–9.
Vivekanadan-Giri A, Wang JH, et al. Mass spectrometric quantification of amino acid oxidation products identifies oxidative mechanisms of diabetic end-organ damage. Rev Endocr Metab Disord. 2008;9(4):275–87.
Vivekanandan-Giri A, Byun J, et al. Quantitative analysis of amino acid oxidation markers by tandem mass spectrometry. Methods Enzymol. 2011;491:73–89.
Wen Y, Gu J, et al. Elevated glucose and diabetes promote interleukin-12 cytokine gene expression in mouse macrophages. Endocrinology. 2006;147(5):2518–25.

Mammalian Target of Rapamycin (mTOR)

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Synonyms

FK506 binding protein 12-rapamycin associated protein 1 (FRAP1); Mammalian target of rapamycin (mTOR); Rapamycin and FK506 binding protein 12 targets 1 (RAFT1)

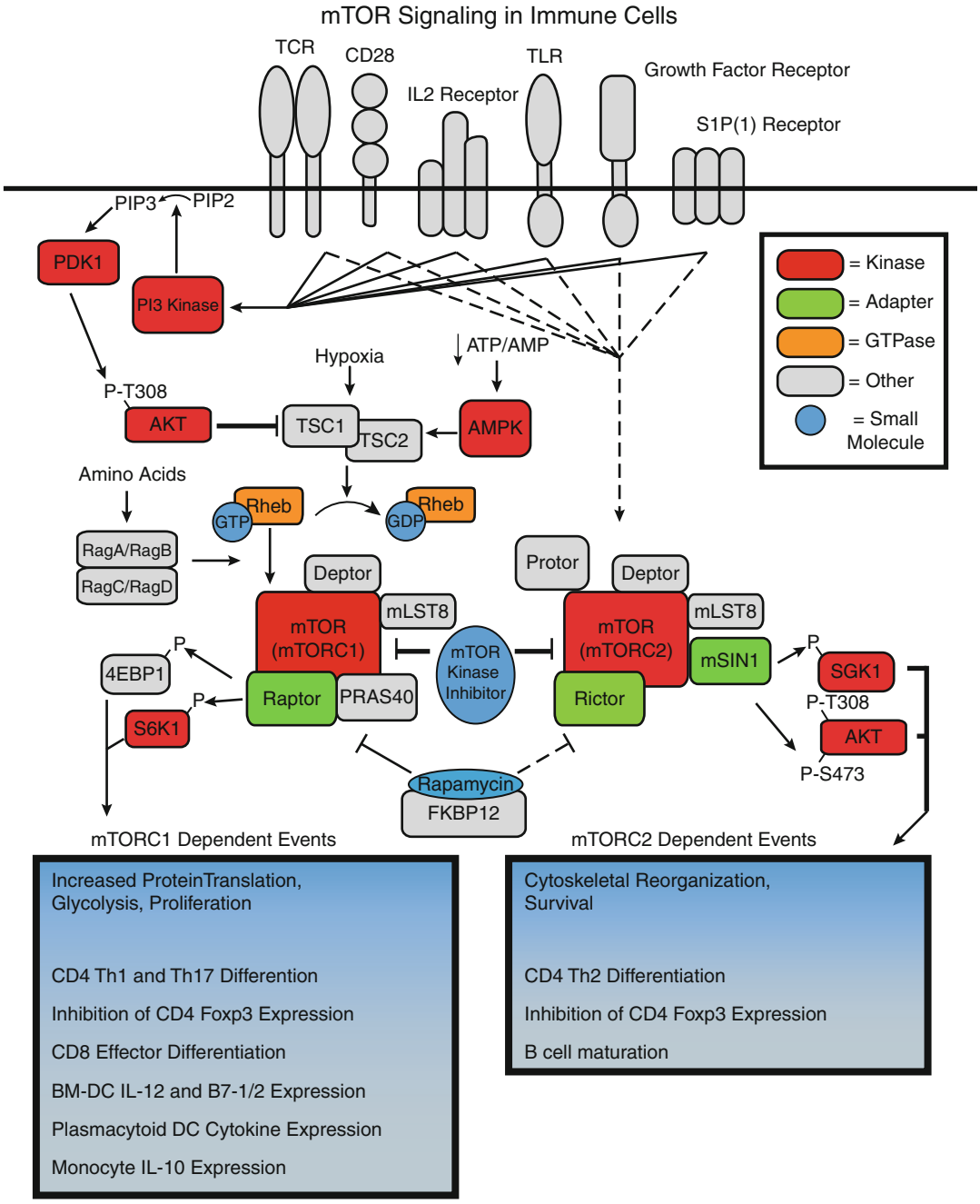
Definition

mTOR is a 289-kD evolutionarily conserved protein with homology to phosphoinositide 3 (PI3)-kinase. Unlike PI3-kinase, mTOR possesses serine-threonine kinase activity sensitive to inhibition by the macrolide antibiotic rapamycin isolated from *Streptomyces hygroscopicus* collected on *Rapa nui* (Easter Island) (reviewed in (Dennis et al. 1999)). The rapamycin-dependent inhibition of kinase activity was used to identify both yeast and mammalian TOR proteins. Rapamycin was quickly noted to have profound immunosuppressive effects, but an understanding of the mechanisms by which mTOR inhibition mediates immunologic tolerance has only recently been more clearly understood.

Yeast have two separate genes, TOR1 which regulates protein translation and TOR2 which regulates cytoskeletal reorganization, while mammalian cells utilize the mTOR protein encoded by the single *Frap1* gene to form two distinct multi-protein complexes termed mTORC1 and mTORC2. Each complex has different inputs for activation and unique downstream substrates and functions (reviewed in (Laplante and Sabatini 2009)). A schematic view of mTOR signaling is depicted in Fig. 1. mTORC1 consists of mTOR, the regulatory-associated protein of mTOR (raptor), the mammalian lethal with Sec13 protein 8 (mLST8), the proline-rich Akt substrate 40 kDa (PRAS40), and the DEP-domain-containing mTOR-interacting protein (Deptor). mTORC2 consists of mTOR, the raptor-independent companion of TOR (rictor), mLST8, the mitogen-activated protein kinase-associated protein 1 (MAPKAP1 also called mSIN1), and the protein observed with rictor (Protor). Inputs that activate mTORC1 include growth factor signaling such as insulin, the presence of adequate cellular energy stores via inhibition of the AMP-activated protein kinase (AMPK) pathway, the presence of sufficient amino acids, the presence of normoxia, and in T cells, activation of CD28 costimulatory molecules and signaling via the interleukin-2 receptor (IL-2R) (reviewed in (Thomson et al. 2009)). Pathways linking several of these inputs to mTORC1 activation include protein kinase B (also called AKT)-dependent phosphorylation of the tuberous sclerosis 1 and 2 protein complex (TSC1/TSC2). The TSC1/2 complex functions as a GTPase-activating protein (GAP) for the GTPase Ras-homolog enriched in brain (Rheb). Phosphorylation of TSC1/2 inhibits GAP activity, increasing Rheb-GTP levels resulting in mTORC1 activation (Laplante and Sabatini 2009). Inputs that activate mTORC2 are less well understood, but like mTORC1, mTORC2 is strongly activated in T cells in the presence of costimulation and cytokine exposure (reviewed in (Cantrell 2002)). mTORC1 is exquisitely sensitive to inhibition by the complex of rapamycin with FKBP12, resulting in loss of phosphorylation of the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and the p70 ribosomal S6 kinase 1 (S6K1) and subsequent inhibition of protein translation (Laplante and Sabatini 2009). Measurement of 4E-BP1 and S6K1 phosphorylation is utilized to monitor the activation status of mTORC1. mTORC2 activity is insensitive to low doses of rapamycin in vitro; however, upon incubation of cells with higher rapamycin doses or following prolonged exposure to rapamycin, loss of mTORC2-dependent AKT S473 phosphorylation is detected (Delgoffe et al. 2011). More recently, small molecules that directly inhibit the kinase activity of mTOR have been developed. These interfere with both mTORC1- and mTORC2-dependent phosphorylation events (Thoreen et al. 2009). Consequences of pharmacologic and genetic blockade of mTOR signaling in various immune cell types are explored below and summarized in Table 1.

mTOR Function in T Cells

Normal T cell activation is dependent on simultaneous recognition of antigen in the form of peptide bound to major histocompatibility complex (MHC) on the antigen-presenting cell (APC) surface by cognate TCR (Signal 1) with concurrent activation of T cell costimulatory molecules including CD28 by APC-expressed B7-1 or B7-2 surface proteins (Signal 2). Such activation leads to T cell interleukin (IL)-2 secretion, autocrine IL-2 stimulation, and proliferation resulting in clonal expansion of the peptide-specific T cell. Biochemically, signals resulting from costimulation, cytokine receptors, and the local nutrient supply are integrated via mTOR signaling in T cells. mTOR activation results in increased expression of metabolic enzymes to permit cell growth and division, as well as acquisition of effector functions such as cytokine expression. Following TCR stimulation in the absence of costimulation (Signal 1 alone), mTOR is not activated, and a T cell is rendered unresponsive to subsequent full stimulation (Signal 1 + 2) in vitro, a condition termed anergy (reviewed in (Powell 2006)). Full stimulation (Signal 1 + 2) in the presence of 2-deoxyglucose



Mammalian Target of Rapamycin (mTOR), Fig. 1 mTOR activation by environmental stimuli: an overview of the mTOR signaling cascade

(2DG) or 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) mimicking energy depletion, N-acetyl-leucine (NALA) mimicking amino acid deprivation, and rapamycin all inhibit normal mTOR activation and result in anergy upon restimulation of the T cell in the absence of the inhibitory drug (Zheng et al. 2009). Importantly, induction of anergy depends on

Mammalian Target of Rapamycin (mTOR), Table 1 Summary of mTOR-dependent signaling in immune cells

Cell type	Experimental manipulation of mTOR	Effect
CD4 T cell	Proximal blockade of mTOR activation (costimulatory blockade, 2-DG, AICAR, NALA)[a], rapamycin, or mTOR kinase inhibitor during T cell activation	Decreased proliferation and cytokine secretion upon restimulation (anergy)
Naïve CD4 T cell	Initial T cell activation in the presence of rapamycin or after genetic inactivation of mTOR	Enhanced expression of FoxP3 and generation of Treg; inhibition of HIF-1α-dependent Th17 differentiation by rapamycin
Naïve CD4 T cell	Thymopoiesis of T cells overexpressing S1P(1) with hyperactivation of mTOR after TCR/CD28 signaling	Inhibition of FoxP3 expression, enhanced Th1 differentiation, spontaneous autoimmunity
Naïve CD4 T cell	Initial T cell activation after genetic inactivation of mTORC1 (via deletion of Rheb)	Inhibition of Th1 and Th17 differentiation with preservation of Th2 differentiation
Naïve CD4 T cell	Initial T cell activation after genetic inactivation of mTORC2 (via deletion of rictor)	Inhibition of Th2 differentiation with preservation of Th17 and preservation or decrease of Th1 and differentiation
Naïve CD8 T cell	Initial T cell activation after genetic inactivation of mTORC1 (via deletion of Rheb)	Decreased CD8 effector gene expression
Naïve CD8 T cell	Initial T cell activation after blockade of mTOR with rapamycin	Enhanced frequency of antigen-specific memory CD8 T cells
Bone marrow-derived dendritic cell	LPS and IL-4 stimulation during blockade of mTOR with rapamycin	Inhibition of costimulatory molecule surface expression and IL-12 secretion (generation of "tolerogenic" DC)
Plasmacytoid dendritic cell	CpG oligonucleotide stimulation during blockade of mTOR with rapamycin	Inhibition of IFN-α, IFN-β, TNF-α, and IL-6 secretion
Monocyte, macrophage, mature dendritic cell	LPS stimulation during blockade of mTOR with rapamycin	Inhibition of IL-10 and enhancement of IL-12 secretion
B cell	Partial blockade of mTOR in hypomorphic knock-in mouse	Partial block in B cell maturation in the bone marrow and reduced numbers of transitional, marginal zone, and follicular B cells in the spleen; decreased T-independent antibody production
B cell	Selective disruption of mTORC2 by deletion of mSin1	Reduction of mature IgM + B cells
B cell	Constitutive activation of mTOR via conditional deletion of TSC1	Decreased B cell maturation and a reduction in marginal zone B cells in the spleen; decreased T-dependent and loss of T-independent antibody production

[a]Abbreviations: *2-DG* 2-deoxyglucose, *AICAR* 5-aminoimidazole-4-carboxamide ribonucleoside, *NALA* N-acetyl leucine

TCR-mediated, calcium-dependent activation of nuclear factor of activated T cells (NF-AT), as demonstrated by the fact that the immunosuppressive drugs cyclosporine or FK506 that block calcium-dependent NF-AT activation actually prevent the induction of anergy by Signal 1 alone or by Signal 1 + 2 in the presence of rapamycin (Powell 2006). Clinical implications of this difference in mechanism of immune modulation between rapamycin and calcineurin inhibitors are discussed below.

Following activation, naïve CD4 T cells acquire specialized effector function in response to the particular cytokine and costimulatory milieu present at the time of activation. Different effector fates are associated with induction of specific master transcriptional regulators and restricted patterns of cytokine expression,

for example, Th1 cells express T-box expressed in T cells (Tbet) and secrete interferon (IFN)-γ, Th2 cells express GATA3 and secrete IL-4, Th17 cells express retinoic acid receptor-related orphan receptor γ (RORγT) and express IL-17A/F, and a subset of CD4 T cells termed Tregs acquires suppressive regulatory function dependent on Forkhead-box-P-3 (Foxp3) transcription factor expression (reviewed in (Weaver et al. 2006)). mTOR plays a critical role in CD4 effector differentiation. Activation of T cells in the presence of rapamycin in vitro increases the frequency of cells expressing Foxp3 that have suppressor function (Battaglia et al. 2005). Genetic blockade of mTOR signaling by conditional deletion of mTOR in T cells using CD4-Cre transgenic mice does not interfere with generation of naïve T cells. However, following activation, mTOR-deficient CD4 T cells upregulate FoxP3 expression, fail to express effector cytokines, and become functional suppressor cells (Delgoffe et al. 2009). Reciprocally, excessive activation of mTOR in mice bearing T cells transgenic for the sphingosine 1-phosphate receptor S1P(1) suppressed generation of normal FoxP3+ Tregs and promoted Th1 effector cytokine expression resulting in spontaneous autoimmunity (Liu et al. 2009). Inhibition of FoxP3 expression downstream of mTOR signaling has been demonstrated to partly result from the induction of hypoxia-induced factor-1 alpha (HIF-1α) expression which enhances Th17 cell generation by cooperating with RORγT to promote IL-17 expression and simultaneously direct proteolytic degradation of FoxP3 protein (Dang et al. 2011).

Somewhat unexpectedly, the enhanced FoxP3 expression in the absence of mTOR is dependent on complete loss of both mTORC1 and mTORC2 signals, as selective deletion of mTORC1 alone via T cell conditional deletion of Rheb results in selective inhibition of Th1 and Th17 cell generation but permits Th2 cells (Delgoffe et al. 2011). Conversely, selective deletion of mTORC2 alone via T cell conditional deletion of rictor results in inhibition of Th2 cells but permits Th17 effectors and has variable effects on Th1 cells in two reports (Delgoffe et al. 2011; Lee et al. 2010). The complete mechanism by which loss of either mTORC1 or mTORC2 selectively impairs cytokine responses of naïve T cells remains under investigation, but mTORC1 null T cells demonstrate decreased Signal Transducers and Activators of Transcription(STAT)4 phosphorylation after stimulation with IL-12 and STAT3 phosphorylation after stimulation with IL-6 (Delgoffe et al. 2011). Variable effects of mTORC2 deletion on cytokine signaling in T cells have been reported, with impaired STAT6 activation after stimulation with IL-4 in rictor-floxed mice on a CD4-Cre background (Delgoffe et al. 2011) but normal IL-4 induced STAT6 phosphorylation with abnormal TCR-induced proximal AKT and distal PKC-θ activation in rictor-floxed mice on the dLck-iCre background (Lee et al. 2010). In summary, while selective loss of mTORC1 or mTORC2 impairs a subset of CD4 effector fates, complete loss of mTOR signaling results in generation of Tregs instead of effector cells.

mTOR has also been shown to participate in generation of effector CD8 T cells. TCR stimulation, IFN-γ signaling, and IL-12 signaling in naïve CD8 cells lead to strong mTORC1 activation and resulting upregulation of Tbet, promoting proliferation and expression of effector genes such as perforin and granzyme B (Rao et al. 2010). CD8 T cells lacking mTORC1 due to deletion of Rheb or with constitutively activated mTORC1 due to deletion of TSC2 have impaired and enhanced CD8 effector function, respectively, consistent with necessity of mTORC1 in activation of the CD8 effector program (Pollizi, K.P. and Powel, J.D., manuscript in preparation). Following control of an acute infection, the number of antigen-specific CD8 T cells declines, and the cells downregulate expression of Tbet and increase expression of a homologous transcription factor eomesodermin (Eomes) along with the IL-7 receptor, CD127, and the IL-2/IL-15 receptor beta chain, CD122, in order to persist as a smaller population of long-lived memory CD8 cells. Somewhat unexpectedly, blockade of mTOR with low-dose rapamycin during acute infection of mice with lymphocyte choriomeningitis virus (LCMV) does not impair the antiviral response; rather, it enhances the

frequency and persistence of virus-specific CD8 T cells (Araki et al. 2009). Furthermore, mTOR inhibition during vaccination has also been shown to enhance antitumor immune responses (Rao et al. 2010). Mechanistically, this appears to be due to effects of rapamycin on promoting Eomes expression at the expense of Tbet, thereby increasing the frequency of memory CD8 cells.

mTOR Function in Antigen-Presenting Cells

Antigen-presenting cells (APCs) are critical bridges between innate immune recognition of infectious pathogens and the adaptive immune response. The cytokines produced by APCs following activation with pathogen-associated molecular patterns (PAMPs) such as bacterial lipopolysaccharide, viral ribonucleic acid, and fungal cell wall components initiate the inflammatory response and recruit adaptive immune cells to fight infection. Following activation, APCs upregulate cell-surface expression of costimulatory molecules including B7-1 and B7-2, resulting in full activation of T cells that recognize antigenic peptides taken up and presented by the APC. Effector differentiation of the activated T cells is then influenced by the local cytokines secreted from the APC. mTOR signaling within the APC regulates many of these critical steps in a manner that is cell type- and activation state-specific.

Resting bone marrow-derived dendritic cells (DCs) are poor stimulators of T cells but acquire potent immune-stimulatory capacity when "matured" following LPS stimulation and culture with IL-4 or CD40 cross-linking as a consequence of IL-12 secretion and induction of surface B7-1/B7-2 expression. Maturation in the presence of rapamycin inhibits both IL-12 and costimulatory molecule expression, rendering the rapamycin-treated DC tolerogenic rather than stimulatory in a manner at least partly dependent on an enhanced ability to induce CD4 T cells to become FoxP3+ Treg (Turnquist et al. 2007). In plasmacytoid dendritic cells, CpG oligonucleotides which mimic viral infection elicit robust secretion of IFN-α, IFN-β, tumor necrosis factor (TNF)-α, and IL-6 in a manner sensitive to inhibition by rapamycin (Cao et al. 2008). In contrast, in monocytes in peripheral blood, macrophages, and mature DCs, mTOR signaling appears to limit secretion of Th1-promoting IL-12 by enhancing secretion of the immunosuppressive cytokine IL-10. Thus, stimulation of these cell types with LPS in the presence of rapamycin has the paradoxical effect of inhibiting IL-10 with resulting enhanced IL-12 secretion and stronger Th1 immune responses in some systems (Weichhart et al. 2008). Which of these two opposing effects of mTOR inhibition on APC function are dominant in vivo appears to depend on the type of inflammatory stimulus and the dose and timing of mTOR inhibition used. For example, rapamycin treatment protects susceptible Balb/C mice from Listeria infection and augments LCMV immunity but impairs immunity to yellow fever vaccine (Araki et al. 2009; Cao et al. 2008; Weichhart et al. 2008).

mTOR Function in B Cells

The PI3-kinase/mTOR axis is activated in B cells following surface immunoglobulin (Ig) cross-linking, LPS stimulation, and CD40 ligation and resulting proliferation and antibody secretion that is inhibited by rapamycin (reviewed in (Thomson et al. 2009)). Several genetic models have very recently been utilized to examine the mechanism for these effects. An mTOR hypomorphic mouse generated by insertion of a neo-cassette within the Frap1 gene was found to have lymphopenia and reduced numbers of both T and B cells, with a partial block in B cell maturation in the bone marrow and reduced numbers of transitional, marginal zone, and follicular B cells in the spleen. These mice make poor responses to the T-independent antigen nitrophenol-LPS (Zhang et al. 2011). Selective disruption of mTORC2 by deletion of mSin1 resulted in a reduction of mature IgM + B cells in irradiated mice reconstituted with mSin1-deficient fetal liver cells. This was due to a partial arrest at the pro-B cell stage associated with persistent Rag1

gene expression and enhanced IL-7 receptor expression. Mechanistically, this was found to be due to enhanced nuclear translocation of the Forkhead family transcription factor FoxO1 as a consequence of reduced mTORC2-dependent FoxO1 phosphorylation that retains FoxO1 in the cytoplasm (Lazorchak et al. 2010). Unlike in T cells, constitutive activation of mTOR via conditional deletion of TSC1 using CD19-Cre did not produce a reciprocal phenotype to the mTOR hypomorph or mTORC2 null mice reported above. Instead, mice with constitutively active mTOR in B cells also have decreased B cell maturation and a reduction in marginal zone B cells in the spleen. This effect was partially rescued by rapamycin treatment. Such mice make poor responses to T-dependent antigen and fail to make a response to a T-independent antigen (Benhamron and Tirosh 2011). While more details regarding the targets of mTOR-dependent signaling in B cells remain to be identified, these early data are consistent with mTOR-dependent signaling being necessary for normal generation of naïve B cells and for regulation of mature B cell proliferation/activation. They also suggest that physiologic limitation of the duration or intensity of mTOR signaling during B cell terminal differentiation may be necessary for to permit normal antibody production during an immune response.

mTOR Inhibition as Clinical Immunosuppressant

Given the pleiotropic effects of mTOR signaling on cells involved in both innate and adaptive immunity, it is not surprising that rapamycin and related compounds have found clinical utility in the fields of solid organ and hematopoietic stem cell transplantation. Numerous examples of both animal models and human systems are reported, but due to space limitations, only a few are highlighted that help to illustrate important differences between tolerogenic mTOR inhibition and conventional calcineurin-based immunosuppression that may interfere with generation of long-term tolerance.

In a murine model of cardiac allograft rejection, DCs were matured in vitro in the presence of rapamycin and infused into cardiac allograft recipients who received a short course of systemic rapamycin. This resulted in long-term allograft survival off immunosuppression, increased allograft infiltration by Foxp3+ Tregs, and the ability to adoptively transfer tolerance to secondary recipients by infusion of CD4 T cells isolated from tolerant graft recipients (Turnquist et al. 2007). Several groups have isolated human Tregs for expansion in vitro, relying on culture with rapamycin to promote sustained Foxp3 expression, with the goal of applying these to treat autoimmune disease or prevent graft versus host disease (GVHD). This technology has been applied in a phase I clinical trial of Treg infusion following unrelated umbilical cord blood transplantation and demonstrated safety and lower rates of GVHD compared to historical controls from the same institution (Brunstein et al. 2011).

Systemic treatment with rapamycin is also used to promote tolerance. The longer clinical experience with calcineurin-based GVHD prophylaxis in hematopoietic stem cell transplant has resulted in most trials adding rapamycin to regimens containing cyclosporine or tacrolimus. Despite the mechanistic antagonism, there is encouraging evidence of a favorable incidence of acute GVHD, as low as 20.5 % Grade II–IV acute GVHD following myeloablative transplant with tacrolimus- and sirolimus-based GVHD prophylaxis (Cutler et al. 2007). However, based on the potential inhibition of rapamycin-induced tolerance by calcineurin inhibitors and preclinical work demonstrating that treatment with low-dose total body irradiation (TBI) and rapamycin alone permitted stable mixed donor chimerism in a murine model of non-myeloablative haploidentical bone marrow transplant, a human clinical trial was opened for the treatment of severe sickle cell anemia with matched sibling donor peripheral blood stem cell transplant using a regimen of alemtuzumab, low-dose TBI, and post-transplant rapamycin. This study achieved stable mixed donor chimerism in 9 of 10 treated patients, no patients experienced acute or chronic GVHD, all had

resolution of sickle cell-related symptoms, and two patients who discontinued rapamycin have remained stable mixed chimeras off of immunosuppression (Hsieh et al. 2009).

In summary, mTOR signaling plays an essential role in integration and transmission of signals in multiple immune cell types leading to cellular activation, proliferation, and effector differentiation. Thoughtful blockade of mTOR signaling appears to offer significant promise as a means to achieve immunologic tolerance in the setting of organ and hematopoietic stem cell transplantation while preserving the ability to make protective immune responses against viruses, bacteria, and tumors.

mTORC1 consists of mTOR, raptor, mLST8, PRAS40, and Deptor. mTORC2 consists of mTOR, rictor, mLST8, Deptor, MAPKAP1 (also called mSIN1), and Protor. In the figure, straight arrows indicate activating signals, and lines ending in a bar indicate inhibitory signals. Curved arrows indicate chemical reactions catalyzed by the indicated enzymes. "P" indicates select sites of protein phosphorylation. Inputs that activate mTORC1 include growth factor signaling such as insulin, the presence of adequate cellular energy stores via activation of the AMP-activated protein kinase (AMPK) pathway, the presence of sufficient amino acids via the Rag (RagA/B/C/D) family of GTPases that promote Rheb colocalization with mTORC1, the presence of normoxia via regulated in development and DNA damage responses 1 (REDD1), and in T cells activation of TCR, CD28 and other costimulatory molecules, sphingosine 1-phosphate receptor 1 (S1P(1) receptor), and signaling via the interleukin-2 receptor (IL-2R). Pathways linking several of these inputs to mTORC1 activation include protein kinase B (also called AKT)-dependent phosphorylation of the tuberous sclerosis 1 and 2 protein complex (TSC1/TSC2). The TSC1/2 complex functions as a GTPase-activating protein (GAP) for the GTPase Ras-homolog enriched in brain (Rheb). Phosphorylation of TSC1/2 inhibits GAP activity, increasing Rheb-GTP levels resulting in mTORC1 activation. Inputs that activate mTORC2 are less well understood, but like mTORC1, mTORC2 is activated in T cells by costimulation and cytokine exposure. mTORC1 is inhibited by the complex of rapamycin with FKBP12, resulting in loss of phosphorylation of the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and the p70 ribosomal S6 kinase 1 (S6K1) and subsequent inhibition of protein translation. Measurement of 4E-BP1 and S6K1 phosphorylation is utilized to monitor the activation status of mTORC1. mTORC2 activity is insensitive to low doses of rapamycin in vitro; however, upon incubation of cells with higher rapamycin doses or following prolonged exposure to rapamycin, loss of mTORC2-dependent AKT S473 phosphorylation is detected. Phosphorylation of both mTORC1 and mTORC2 substrates is blocked by small molecule inhibitors of mTOR kinase activity. Boxes at the bottom catalogue cellular responses known to be downstream of mTORC1 or mTORC2. Global functions ascribed to each pathway are listed at the top in shaded areas, and the growing list of functions defined in immune cells is listed at the bottom.

Cross-References

- ▶ B7 and CD28 Families
- ▶ Cytotoxic T Lymphocytes
- ▶ PI3K

References

Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, Larsen CP, Ahmed R. mTOR regulates memory CD8 T-cell differentiation. Nature. 2009;460:108–12.

Battaglia M, Stabilini A, Roncarolo MG. Rapamycin selectively expands CD4 + CD25 + FoxP3+ regulatory T cells. Blood. 2005;105:4743–8.

Benhamron S, Tirosh B. Direct activation of mTOR in B lymphocytes confers impairment in B-cell maturation and loss of marginal zone B cells. Eur J Immunol. 2011;41:2390–6.

Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, Defor T, Levine BL, June CH, Rubinstein P, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. Blood. 2011;117:1061–70.

Cantrell D. Protein kinase B (Akt) regulation and function in T lymphocytes. Semin Immunol. 2002;14:19–26.

Cao W, Manicassamy S, Tang H, Kasturi SP, Pirani A, Murthy N, Pulendran B. Toll-like receptor-mediated induction of type I interferon in plasmacytoid dendritic cells requires the rapamycin-sensitive PI(3)K-mTOR-p70S6K pathway. Nat Immunol. 2008;9:1157–64.

Cutler C, Li S, Ho VT, Koreth J, Alyea E, Soiffer RJ, Antin JH. Extended follow-up of methotrexate-free immunosuppression using sirolimus and tacrolimus in related and unrelated donor peripheral blood stem cell transplantation. Blood. 2007;109:3108–14.

Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, Fu J, Kim Y, Yen HR, et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell. 2011;146:772–84.

Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, Worley PF, Kozma SC, Powell JD. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity. 2009;30:832–44.

Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, Xiao B, Worley PF, Powell JD. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat Immunol. 2011;12:295–303.

Dennis PB, Fumagalli S, Thomas G. Target of rapamycin (TOR): balancing the opposing forces of protein synthesis and degradation. Curr Opin Genet Dev. 1999;9:49–54.

Hsieh MM, Kang EM, Fitzhugh CD, Link MB, Bolan CD, Kurlander R, Childs RW, Rodgers GP, Powell JD, Tisdale JF. Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. N Engl J Med. 2009;361:2309–17.

Laplante M, Sabatini DM. mTOR signaling at a glance. J Cell Sci. 2009;122:3589–94.

Lazorchak AS, Liu D, Facchinetti V, Di Lorenzo A, Sessa WC, Schatz DG, Su B. Sin1-mTORC2 suppresses rag and il7r gene expression through Akt2 in B cells. Mol Cell. 2010;39:433–43.

Lee K, Gudapati P, Dragovic S, Spencer C, Joyce S, Killeen N, Magnuson MA, Boothby M. Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. Immunity. 2010;32:743–53.

Liu G, Burns S, Huang G, Boyd K, Proia RL, Flavell RA, Chi H. The receptor S1P1 overrides regulatory T cell-mediated immune suppression through Akt-mTOR. Nat Immunol. 2009;10:769–77.

Powell JD. The induction and maintenance of T cell anergy. Clin Immunol. 2006;120:239–46.

Rao RR, Li Q, Odunsi K, Shrikant PA. The mTOR kinase determines effector versus memory CD8+ T cell fate by regulating the expression of transcription factors T-bet and Eomesodermin. Immunity. 2010;32:67–78.

Thomson AW, Turnquist HR, Raimondi G. Immunoregulatory functions of mTOR inhibition. Nat Rev Immunol. 2009;9:324–37.

Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS. An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem. 2009;284:8023–32.

Turnquist HR, Raimondi G, Zahorchak AF, Fischer RT, Wang Z, Thomson AW. Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic CD4+ T cells, but enrich for antigen-specific Foxp3+ T regulatory cells and promote organ transplant tolerance. J Immunol. 2007;178:7018–31.

Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity. 2006;24:677–88.

Weichhart T, Costantino G, Poglitsch M, Rosner M, Zeyda M, Stuhlmeier KM, Kolbe T, Stulnig TM, Horl WH, Hengstschlager M, et al. The TSC-mTOR signaling pathway regulates the innate inflammatory response. Immunity. 2008;29:565–77.

Zhang S, Readinger JA, DuBois W, Janka-Junttila M, Robinson R, Pruitt M, Bliskovsky V, Wu JZ, Sakakibara K, Patel J, et al. Constitutive reductions in mTOR alter cell size, immune cell development, and antibody production. Blood. 2011;117:1228–38.

Zheng Y, Delgoffe GM, Meyer CF, Chan W, Powell JD. Anergic T cells are metabolically anergic. J Immunol. 2009;183:6095–101.

Marginal Zone B Cells

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Synonyms

$CD27^+$ IgM^+ IgD^+ B cells; IgM memory B cells

Definition

Marginal zone (MZ) B cells are a subpopulation of B lymphocytes that localize at the border of the red and white pulp in the spleen where they are positioned to sample blood-borne antigens. Accordingly, MZ B cells mount rapid and T cell–independent antibody responses to blood-borne pathogens that display repetitive epitopes such as the polysaccharides found on

encapsulated bacteria and viral coat proteins. They are derived as a separate lineage from naïve follicular B cells and express a unique phenotype. The antibodies produced by MZ B cells bear little-to-no mutation but often display polyreactive and auto-reactive specificities.

Introduction

Antibodies are a critical component of adaptive immunity and their production is a cooperative effort between different B cell subpopulations (Swanson et al. 2013). MZ B cells contribute to humoral immunity upon pathogen infection by rapidly producing pathogen-specific antibodies that limit and contain infection and during the time needed for the generation of somatically mutated high affinity antibodies of appropriate subclass that are produced by naïve follicular B cells (Cerutti et al. 2013; Martin and Kearney 2002; Pillai et al. 2005; Weill et al. 2009). MZ B cell antibody responses are typically comprised of IgM and limited IgG subclass (IgG2 in humans and IgG3 in mice) antibodies that harbor little-to-no mutations and thus are of relatively weak affinity. However, these pathogen-specific antibodies are effective due to the avidity provided by the pentameric IgM and because the MZ B cell–derived antibodies often display polyreactive specificities that include determinants common to many microbial pathogens.

Much of our understanding about the development and function of MZ B cells comes from rodent studies performed over the last several decades (Martin and Kearney 2002; Pillai et al. 2005). However, human and murine MZ B cells are analogous populations that share many similar features that include phenotype, localization in lymphoid organs and ability to respond to pathogenic encapsulated bacteria in a T cell–independent manner (Cerutti et al. 2013; Weill et al. 2009).

Phenotype

The MZ B cells in both humans and mice express a very similar pattern and level of receptors defined as IgM^{hi} IgD^{lo} $CD21^{hi}$ $CD1^{hi}$ $CD23^{lo}$, presumably reflecting a similar function in both species. Human MZ B cells additionally express CD27, a surface antigen previously considered to be a marker of memory B cells (Klein et al. 1998). In contrast to murine MZ B cells, whose antibodies are germline encoded, human MZ B cells harbor (relatively few) somatic mutations in their immunoglobulin (Ig) genes (Cerutti et al. 2013; Weill et al. 2009). These features led to their initial consideration as "IgM memory" B cells (Klein et al. 1998). However, introduction of somatic mutations and production of memory B cells occurs in germinal centers, and mutated IgM^{+} IgD^{+} $CD27^{+}$ human B cells are nevertheless present in individuals with mutations that preclude germinal center formation (Weill et al. 2009). Furthermore, IgM^{+} IgD^{+} $CD27^{+}$ human B cells function analogously to murine MZ B cells (Kruetzmann et al. 2003; Weller et al. 2004). Thus, $CD27^{+}$ human MZ B cells are functionally analogous to murine MZ B cells and are not germinal-center experienced conventional memory B cells.

Human and mouse MZ B cells are larger cells compared to naïve follicular B cells. In the mouse, these large MZ B cells are considered "pre-activated" as they display elevated levels of activation antigens such as CD86 and have a lower threshold of activation by either specific antigen or toll-like receptor ligands (Capolunghi et al. 2008; Snapper et al. 1993). The antibody repertoire expressed by both mouse and human MZ B cells also shares a number of features and differs from the major follicular B cell population. Notably, the murine MZ B cell antibody repertoire is known to harbor polyreactive specificities that include weak reactivity to autoantigens and that are important in promoting the development of the MZ B cell population (Martin and Kearney 2002; Pillai et al. 2005). Consistent with this, MZ B cell antibodies from humans and mice also harbor relatively short complementary determining region 3 (CDR3) Ig heavy chain sequences (Briney et al. 2012; Schelonka et al. 2007) that are also a common feature of antibodies with polyreactive specificities (Chen et al. 1991). Finally, human MZ

B cells recirculate and, thus, are present in blood and other lymphoid organs where they reside in microenvironments among macrophages similar to those present in the marginal zone of the spleen. Murine MZ B cells do not recirculate and are restricted to the spleen. However, they are not restricted to the marginal zone as they continuously shuttle antigen into the B cell follicle to deliver it to follicular dendritic cells (Cyster and Schwab 2012).

Development

MZ B cells in the mouse develop from bone marrow–derived transitional B cells as a separate lineage from the major follicular B cell population in a process that is influenced by both antigen specificity and signaling ability of the B cell antigen receptor (BCR) (Martin and Kearney 2002; Pillai et al. 2005). The precise signals that dictate development of immature B cells into FO or MZ B cells are unknown, but certain specificities provided by Ig transgenes promote or discourage MZ B cell development in mouse models. In addition, a number of studies have suggested that a weak BCR signaling leads to MZ B cell preferential development, as shown in mice with deficient BCR signaling components that include the Btk tyrosine kinase and surface CD19 antigen (Martin and Kearney 2002; Pillai et al. 2005). In addition, both Notch- and Pyk2-deficient mice have suggested that both these signaling molecules are important for MZ B cell development. Whether human MZ B cells also develop as a separate lineage from immature bone marrow B cells similar to their mouse counterparts is not established but their development appears to depend on TLR signaling (Weller et al. 2012). Furthermore, in contrast to mouse MZ B cells, human MZ B cells harbor somatic mutations within their Ig genes that are present even in the absence of germinal center formation. It has been postulated that human MZ B cells introduce somatic mutations during their development independent of antigen and in a mechanism that diversifies the MZ B cell antibody repertoire similar to other species (Weill et al. 2009).

Finally, the development and maintenance of both mouse and human MZ B cells is dependent on the spleen. B cells with a mature MZ B cell phenotype are not present in the spleen of children until approximately 2 years of age or mice until approximately 3–4 weeks of age (Cerutti et al. 2013; Weill et al. 2009). This neonatal lack of MZ B cells is thought to account for the poor antibody responses to encapsulated bacteria by children and neonatal mice.

Localization

In both humans and rodents, MZ B cells reside within the marginal zone that lies at the border between the red and white pulp. In mice, the marginal zone is separated from the white pulp by a marginal sinus into which arterial blood empties, promoting MZ B cell sampling of blood-borne antigens (Mebius and Kraal 2005). In humans, the marginal sinus within the marginal zone appears to be replaced by a plexus of capillaries that terminate in the perifollicular region surrounding the marginal zone (Steiniger et al. 2011). However, the juxtaposition of the marginal zone to this region presumably allows human MZ B cells to similarly sample blood antigens. Importantly, as the marginal zone is juxtaposed to the site where arterial blood empties into the spleen, the marginal zone also harbors post-germinal center memory B cells.

Studies in the mouse have demonstrated that MZ B cell localization in the marginal zone is a result of a signaling balance between chemokine and sphingosine-1-phosphate G-protein coupled receptors (GPCR) and that the $\alpha L\beta 2$ and $\alpha 4\beta 1$ integrins mediate MZ B cell adhesion to their respective ICAM and VCAM ligands expressed by cells within the marginal zone (Cyster and Schwab 2012).

Participation in the Immune Response

MZ B cells are best characterized to mount rapid antibody responses independent of T cells and directed against repetitive surface epitopes on

blood-borne pathogens referred to as T cell–independent type 2 (TI-2) antigens. Physiological TI-2 antigens to which MZ B cells are known to respond include the polysaccharide capsules of pathogenic bacteria such as *Streptococcus pneumoniae*, *Hemophilus influenzae*, and *Neisseria meningitides* (Kruetzmann et al. 2003; Martin and Kearney 2002) and the viral coat proteins of polyoma virus, vesicular stomatitis virus, and foot and mouth disease virus (Gatto and Bachmann 2005). The ability of MZ B cells to rapidly respond to these pathogens is, in large part, due to their location in the spleen within the marginal zone, an area close to the interface between the red and white pulp and where arterial blood is emptied and where in collaboration with specific macrophages, its contents are efficiently sampled. In contrast to germinal center–derived antibody responses, which take time to develop but are somatically mutated and highly specific, MZ B cell antibody responses are rapid and display relatively weak antigen affinity and can be polyreactive.

A notable feature of human MZ B cells is the presence of somatic mutations in Ig genes, and these mutations are introduced during development and independent of germinal centers (Weill et al. 2009). Accordingly, the anti-polysaccharide antibodies produced by human MZ B cells are typically mutated (Zhou et al. 2002). In contrast, abundant evidence from mouse studies suggests that the MZ B cell antibody response to TI-2 antigens is devoid of mutations and, consequently, it is presumed that MZ B cells do not initiate germinal centers. However, it is now clear that the model antigens used to define the MZ B cell antibody response in rodents (typically hapten-coupled polysaccharides) would not be able to engage $CD4^+$ T cells nor facilitate germinal center formation. Thus, the ability of MZ B cells to mount a mutated antibody response to *bona fide* TI-2 antigens, such as those associated with microbial pathogens, may be underappreciated. Indeed, when directly tested mouse MZ B cells are able to contribute to the antibody response against T cell–dependent (TD) protein antigens by seeding germinal centers and undergoing somatic hypermutation (Song and Cerny 2003).

CD21 is expressed at relatively high levels by both human and mouse MZ B cells, and serves not only as a costimulatory molecule but also as a receptor for complement components, thus allowing for the capture of complement-bound antigen. MZ B cells also express nonclassical major histocompatibility complexes (MHC) such as CD1d in rodents and CD1c in humans that allow these cells to interact with a wider range of T cells, including iNKT cells. Finally, MZ B cells can affect more long-term responses by shuttling antigen into the follicle for delivery to follicular dendritic cells (FDCs), which mediate selection during germinal center reactions (Cyster and Schwab 2012).

Conclusion

MZ B cells fill an important niche in humoral immunity by their ability to rapidly produce antibodies against blood-borne pathogen and independent of T lymphocytes. This antibody response contributes to limiting infection during the time needed for highly specific antibodies of appropriate subclass to be generated in germinal centers. A number of features contribute to the expedited MZ B cell antibody response including their localization in the marginal zone of the spleen that facilitates blood sampling, their expressed antibody repertoire that often is capable of recognizing common microbial determinants, and the decreased threshold necessary to be activated.

Cross-References

▶ BCR Signaling
▶ Regulatory B cells

References

Briney BS, Willis JR, McKinney BA, Crowe Jr JE. High-throughput antibody sequencing reveals genetic evidence of global regulation of the naive and memory repertoires that extends across individuals. Genes Immun. 2012;13(6):469–73. doi:10.1038/gene.2012.20.

Capolunghi F, Cascioli S, Giorda E, Rosado MM, Plebani A, Auriti C, et al. CpG drives human transitional B cells to terminal differentiation and production of natural antibodies. J Immunol. 2008;180(2):800–8.

Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nat Rev Immunol. 2013;13(2):118–32. doi:10.1038/nri3383.

Chen C, Stenzel-Poore MP, Rittenberg MB. Natural auto- and polyreactive antibodies differing from antigen-induced antibodies in the H chain CDR3. J Immunol. 1991;147(7):2359–67.

Cyster JG, Schwab SR. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. Annu Rev Immunol. 2012;30:69–94. doi:10.1146/annurev-immunol-020711-075011.

Gatto D, Bachmann MF. Function of marginal zone B cells in antiviral B-cell responses. Crit Rev Immunol. 2005;25(4):331–42.

Klein U, Rajewsky K, Kuppers R. Human immunoglobulin (Ig)M + IgD + peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. J Exp Med. 1998;188(9):1679–89.

Kruetzmann S, Rosado MM, Weber H, Germing U, Tournilhac O, Peter HH, et al. Human immunoglobulin M memory B cells controlling Streptococcus pneumoniae infections are generated in the spleen. J Exp Med. 2003;197(7):939–45. doi:10.1084/jem.20022020.

Martin F, Kearney JF. Marginal-zone B cells. Nat Rev Immunol. 2002;2(5):323–35. doi:10.1038/nri799.

Mebius RE, Kraal G. Structure and function of the spleen. Nat Rev Immunol. 2005;5(8):606–16. doi:10.1038/nri1669.

Pillai S, Cariappa A, Moran ST. Marginal zone B cells. Annu Rev Immunol. 2005;23:161–96. doi:10.1146/annurev.immunol.23.021704.115728.

Schelonka RL, Tanner J, Zhuang Y, Gartland GL, Zemlin M, Schroeder Jr HW. Categorical selection of the antibody repertoire in splenic B cells. Eur J Immunol. 2007;37(4):1010–21. doi:10.1002/eji.200636569.

Snapper CM, Yamada H, Smoot D, Sneed R, Lees A, Mond JJ. Comparative in vitro analysis of proliferation, Ig secretion, and Ig class switching by murine marginal zone and follicular B cells. J Immunol. 1993;150(7):2737–45.

Song H, Cerny J. Functional heterogeneity of marginal zone B cells revealed by their ability to generate both early antibody-forming cells and germinal centers with hypermutation and memory in response to a T-dependent antigen. J Exp Med. 2003;198(12):1923–35. doi:10.1084/jem.20031498.

Steiniger B, Bette M, Schwarzbach H. The open microcirculation in human spleens: a three-dimensional approach. J Histochem Cytochem. 2011;59(6):639–48. doi:10.1369/0022155411408315.

Swanson CL, Pelanda R, Torres RM. Division of labor during primary humoral immunity. Immunol Res. 2013;55((1–3)):277–86. doi:10.1007/s12026-012-8372-9.

Weill JC, Weller S, Reynaud CA. Human marginal zone B cells. Annu Rev Immunol (Rev). 2009;27:267–85. doi:10.1146/annurev.immunol.021908.132607.

Weller S, Braun MC, Tan BK, Rosenwald A, Cordier C, Conley ME, et al. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. Blood. 2004;104(12):3647–54. doi:10.1182/blood-2004-01-0346.

Weller S, Bonnet M, Delagreverie H, Israel L, Chrabieh M, Marodi L, et al. IgM + IgD + CD27+ B cells are markedly reduced in IRAK-4-, MyD88-, and TIRAP-but not UNC-93B-deficient patients. Blood. 2012;120(25):4992–5001. doi:10.1182/blood-2012-07-440776.

Zhou J, Lottenbach KR, Barenkamp SJ, Lucas AH, Reason DC. Recurrent variable region gene usage and somatic mutation in the human antibody response to the capsular polysaccharide of Streptococcus pneumoniae type 23 F. Infect Immun. 2002;70(8):4083–91.

Mechanisms of Endothelial Activation

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Synonyms

Endothelial cell activation; Endothelial dysfunction; Vascular endothelium

Definition

The vascular endothelium is a multifunctional organ that actively participates in the maintenance of vascular homeostasis. Proper endothelial function is critical for vascular health, and endothelial dysfunction causes or contributes to numerous diseases. *Endothelial activation* is a type of endothelial dysfunction that describes the process by which blood-borne and environmental stimuli cause endothelial cells to undergo dramatic functional changes.

Introduction

The vascular endothelium is a monolayer of cells that lines the entire luminal surface of the vasculature and forms a regulatory interface between circulating blood components and underlying tissue compartments. The vascular endothelium covers a network of blood vessels that exceeds 100,000 km in aggregate length, with a surface area of approximately 5,000 m^2 (De Caterina et al. 2007). Its massive size and distribution into all organs and tissues gives the endothelium the capacity to monitor physiological perturbations throughout the entire body. By virtue of its juxtaposition with circulating blood components, the endothelium is critically situated to sense and respond to blood-borne stimuli on a systemic scale, making it the body's largest homeostatic organ (DiCorleto and Fox 2005).

Although it was originally considered a passive barrier, modern research has uncovered numerous mechanisms by which the endothelium actively maintains vascular homeostasis. Under physiological conditions, the endothelium prevents vasospasm, resists leukocyte adhesion, inhibits smooth muscle cell proliferation, and acts as a non-thrombogenic surface for the entire circulatory system. In addition, the endothelium acts as a selective physical barrier by actively regulating the passage of macromolecules and leukocytes into and out of the bloodstream (Pober and Sessa 2007). These physiological roles are influenced by environmental stimuli that can cause the endothelial phenotype to fluctuate between antagonistic functions of vascular homeostasis. At different times or locations, the endothelium can be nonadhesive or hyperadhesive to leukocytes, can be procoagulatory or anticoagulatory, and act as a vasoconstrictor or vasodilator (DiCorleto and Fox 2005). In this way, the endothelium displays a remarkable phenotypic plasticity to adapt to environmental changes. These phenotypic changes are usually normal, adaptive responses to the changing vascular environment, and are an essential contribution to vascular homeostasis.

Localized, nonadaptive changes to endothelial physiology can also alter its phenotype to induce changes in vessel tone, leukocyte adhesiveness, coagulation, and the production of autocrine and paracrine factors, including vasorelaxants and vasospastic substances. These aberrant alterations are collectively referred to as *endothelial dysfunction*, and are involved in the initiation and progression of numerous cardiovascular diseases. *Endothelial activation*, a type of endothelial dysfunction, is a distinct term that describes the process by which blood-borne and environmental stimuli cause endothelial cells to undergo dramatic functional changes and acquire new physiological properties (De Caterina et al. 2007).

Proper endothelial function is critical for maintaining vascular health, and endothelial dysfunction may cause or contribute to numerous vascular diseases. This entry will provide a brief overview of normal endothelial physiology that will serve as a foundation for an in-depth explanation of the molecular mechanisms underlying endothelial cell activation as well as the contribution of endothelial cell activation to the genesis and progression of vascular diseases.

Normal Vascular Endothelium

Morphology and Barrier Function

As a continuous monolayer of cells, the vascular endothelium forms an extensive regulatory interface between the bloodstream and underlying tissues with cells that are elongated in the direction of blood flow (Chiu and Chien 2011).

Abundant intercellular junctions including tight junctions, gap junctions, and adherens junctions allow the endothelium to act as an active physical barrier. The relative abundance of these junctions varies between anatomic locations and confers site-specific specialization of endothelial permeability (Pober and Sessa 2007). Arteries and blood vessels of the brain contain more tight junctions, which restrict macromolecular flux between intravascular and extravascular compartments. Gap junctions, which consist of connexin proteins, link the cytoplasm of neighboring endothelial cells and likely play roles in intercellular communication. Adherens junctions, which consist of cadherin proteins, are important for endothelial cell organization, growth, and migration (De Caterina et al. 2007).

The modulation of intercellular junctions via posttranslational modifications of junction proteins plays a role in the regulation of vascular permeability. The mechanisms vary according to stimuli, but permeability is usually increased by phosphorylation of junction proteins, actin-myosin-dependent morphological changes, and an increased number of intercellular gaps in the endothelial monolayer (Pober and Sessa 2007). Under physiological conditions, these changes are controlled and reversible, representing adaptive control of macromolecular flux between the bloodstream and tissues.

Regulation of Vascular Tone

The endothelium acts as a regulator of vascular tone by synthesizing and secreting vasodilatory or vasoconstrictive substances that act on underlying smooth muscle cells. The most important endothelial-derived vasorelaxing factor is nitric oxide (NO), which is synthesized by endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS). NO exerts a local vasorelaxing effect by diffusing into the smooth muscle cell and activating guanylate cyclase, thereby increasing levels of cyclic guanosine monophosphate (cGMP). Higher levels of cGMP inhibit calcium entry into the smooth muscle cell, resulting in decreased vasoconstriction (Kinlay and Ganz 2007; Lüscher et al. 2005). Endothelial cells are also capable of synthesizing vasoactive metabolites of arachidonic acid called eicosanoids. The most important eicosanoid for maintaining physiological vessel tone is the vasorelaxant prostacyclin. Endothelial cells express the enzymes cyclooxygenase-1,2 (COX-1, COX-2) which synthesize prostaglandin H2 (PGH2) from arachidonic acid (Lüscher et al. 2005). PGH2 can then be converted to a number of vasoactive metabolites, but endothelial cells express significant levels of the enzyme prostacyclin synthase, thus favoring the formation of prostacyclin. Prostacyclin is secreted by endothelial cells and is a potent vasodilatory substance which acts on smooth muscle cells to decrease vascular tone (DiCorleto and Fox 2005).

NO and prostacyclin antagonize a number of endothelial-derived vasoconstrictive substances. These substances, which include angiotensin II, endothelin-1 (ET-1), and platelet-derived growth factor (PDGF), act as agonists of smooth muscle cell contraction (DiCorleto and Fox 2005; Kinlay and Ganz 2007). All three of these molecules are expressed as propeptides by normal endothelial cells until appropriate stimuli trigger their activation by proteolytic cleavage. So, whereas the expression of vasorelaxing substances NO and prostacyclin is normally constitutive, the activation of endothelial-derived vasoconstrictive substances is rapidly inducible, allowing the endothelium to quickly and reversibly regulate vascular tone (De Caterina et al. 2007).

Regulation of Hemostasis

Under normal conditions, the endothelium actively participates in the prevention of blood clot formation. This non-thrombogenic property is maintained by the production of several endothelial-derived factors that act on platelets and enzymes of the coagulation cascade. Endothelial cells synthesize thrombomodulin which binds to thrombin and inactivates thrombin's procoagulant activity (DiCorleto and Fox 2005). Additionally, endothelial cells synthesize the arachidonic acid metabolite prostacyclin, which was previously discussed in terms of its role as a vasodilator. Prostacyclin is a particularly potent inhibitor of platelet aggregation and

platelet adherence to the endothelium. Under physiological conditions, endothelial-derived prostacyclin activates the prostacyclin receptor IP_1 on platelets to inhibit aggregation and adhesion (DiCorleto and Fox 2005).

Endothelial production of prostacyclin, thrombomodulin, and other anticoagulant factors appears to be constitutive under normal conditions, necessitating the rapid production of the procoagulant factors when clotting is required. The endothelium synthesizes numerous thrombotic factors including tissue factor (TF), platelet-activating factor (PAF), and von Willebrand factor (vWF) (DiCorleto and Fox 2005). TF is particularly important for the transformation of the vessel wall into a thrombogenic surface because it promotes the activation of factors X and IX of the coagulation cascade (De Caterina et al. 2007). Healthy vessels maintain a strict balance between thrombotic and antithrombotic factors, favoring the expression of antithrombotic factors under physiological conditions and rapidly producing TF and other coagulant factors following exposure to thrombotic stimuli.

Transduction of Biomechanical Forces

The endothelium is situated in direct contact with circulating blood. As a result, endothelial cells are constantly exposed to a variety of hemodynamic forces including shear stress, hydrostatic pressure, and cyclic strain. Shear stress is particularly significant because it is transduced by endothelial cell-surface proteins to activate signaling pathways that regulate changes in cell morphology, growth, and gene expression (Hahn and Schwartz 2009). The endothelial response to shear stress depends on the type and rate of flow. For example, exposure of endothelial cells to steady laminar flow may induce cell-cycle arrest in G_0 or G_1, whereas exposure to disturbed blood flow exhibits pro-proliferative effects (Chiu and Chien 2011). Additionally, laminar flow causes endothelial cells to become aligned and elongated in the direction of flow, while disturbed flow causes endothelial cells to adopt a more polygonal shape without a clear orientation (Chiu and Chien 2011). Rearrangement of intercellular junctions and cytoskeletal proteins underlies the morphological changes of endothelial cells in response to both types of flow.

Fluid shear stress can also modulate endothelial gene expression. Shear stress response elements (SSRE) have been discovered in the promoters of many genes and act as critical *cis*-acting elements for shear stress-regulated gene expression (DiCorleto and Fox 2005). Mechanotransducers at the endothelial cell surface respond to shear forces to activate signaling pathways and transcription factors that regulate gene induction. The exact mechanisms by which endothelial cell-surface proteins transduce mechanical forces are not known, but cytoskeletal proteins, receptor tyrosine kinases (RTKs), G-protein-coupled receptors (GPCRs), and ion channels all play a role (Hahn and Schwartz 2009). The ability of endothelial cells to react to specific types of biomechanical forces emphasizes its role as a type of organism-wide sensory organ, capable of detecting and responding to a variety of hemodynamic forces depending on both the type and magnitude of the force.

Mechanisms of Endothelial Activation

The original models of the endothelium's role in disease progression were largely based on the response-to-injury hypothesis. This hypothesis stated that the endothelium was a protective, passive barrier whose absence following injury led to abnormal contact between circulating platelets and thrombogenic basement membrane. However, after researchers observed that the endothelium could interact with circulating monocytes, oxidize low-density lipoprotein, and synthesize growth factors and cytokines, it was suggested that the endothelium may play an active role in the development of atherosclerosis and other vascular diseases (DiCorleto and Chisolm 1986). After decades of research, it is well accepted that endothelial activation is associated with a number of vascular diseases including atherosclerosis.

Inflammatory and pro-thrombotic substances transition the endothelium to an activated state

characterized by increased intimal permeability, expression of leukocyte adhesion factors, and shifts in protein secretion. Alterations in the secretion profile affect the balance of pro- and antithrombotic factors, pro- and anti-inflammatory cytokines, growth factors and inhibitors, and vasodilatory and constrictive substances. Endothelial reactions to stimulation are largely coordinated by a central signaling pathway, NF-κB. Stimulation of this pathway results in the induction of hundreds of genes associated with the activated phenotype. The results of endothelial activation are manifested as altered interactions between the endothelium, other cells of the vascular wall, and components of the blood.

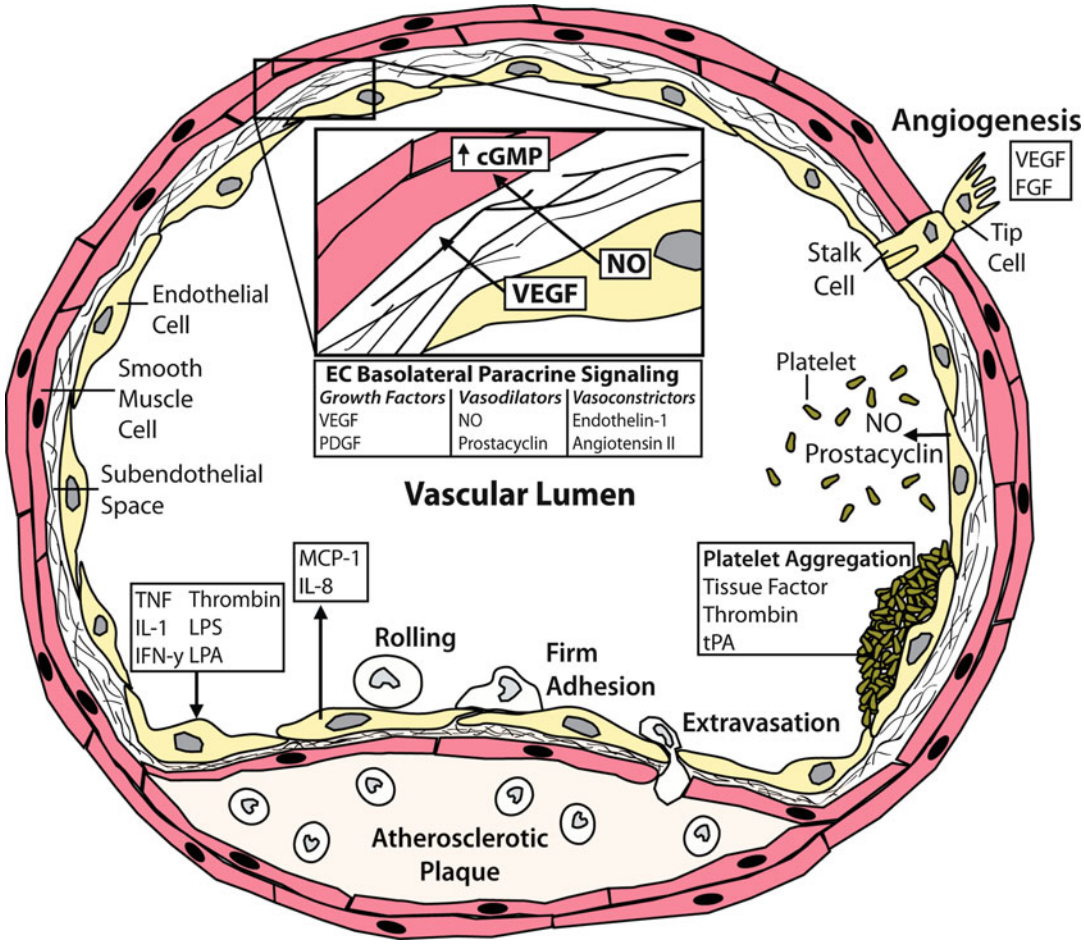
NF-κB as a Central Regulator of Endothelial Cell Activation

The endothelium responds to inflammatory agonists primarily by inducing genes not transcribed in the resting state. Signals for a variety of activating agonists, such as TNF, IL-1, LPS, or oxidized LDL, converge on the NF-κB signaling pathway to activate transcription of target genes (De Caterina et al. 2007). The NF-κB family includes five transcription factors, RelA (p65), RelB, c-Rel, NF-κB1 (p50), and NF-κB2 (p52), and their inhibitors, the IκB subunits, all of which are ubiquitously expressed in mammalian cells. Characteristic of NF-κB family members is a common domain essential for dimerization and DNA binding, the Rel homology domain. At rest, the transcription factors are sequestered by their inhibitory partners in the cytosol. These inhibitors are phosphorylated, usually by the IκB kinase complex (IKK), ubiquitinated, and targeted for proteasomal degradation upon stimulation. Newly liberated NF-κB factors dimerize and translocate to the nucleus, where they bind the κB sequence in the promoter of target genes. The transcriptional activity of NF-κB members is further regulated through associations with coactivators and through a variety of posttranslational modifications (Oeckinghaus et al. 2011). The NF-κB binding site is a remarkably loose, decameric sequence written as GGGRNNTYCC (R = G or A, Y = C or T, N = any nucleotide) (Baltimore 2011). Genes important in the inflammatory program often are induced under the control of NF-kB. These include the adhesion molecules E-selectin, VCAM-1 and ICAM-1, as well as IL-1, IL-6, IL-8, MCP-1, TF, PAI-1, COX-2, and iNOS (De Caterina et al. 2007).

Expression of Leukocyte Adhesion Molecules, Cytokines, and Chemokines

Leukocytes do not interact with healthy, unstimulated endothelial cells, but following exposure to an activating agent, adhesion molecules are expressed on the luminal surface and chemokines are secreted into the blood to interact with and recruit leukocytes (Fig. 1, bottom). Initial interactions between endothelial cells and leukocytes involve the immune cell rolling along the endothelium, followed by arrest, focal adhesion, spreading, and emigration of the leukocyte from the lumen across the endothelial barrier.

Leukocyte rolling on the endothelial surface is a relatively weak interaction governed by the selectin family of proteins, P-, E-, and L-selectin. P-selectin is present in platelet secretory granules, as well as the Weibel-Palade bodies of endothelial cells, and is therefore rapidly displayed on the endothelial cell surface within minutes of agonist exposure (De Caterina et al. 2007; Pober and Sessa 2007). L-selectin is expressed on leukocyte populations and has affinity for ligands expressed on endothelial cells and other leukocytes. E-selectin is only expressed in activated endothelial cells. Stronger interactions mediated by the immunoglobulin superfamily tether the rolling leukocyte to the endothelium. These adhesion molecules include ICAM-1, ICAM-2, and ICAM-3, VCAM-1, and PECAM-1. While low levels of ICAM-1 are constitutively expressed by endothelial cells, this gene and other members of the immunoglobulin family are induced upon activation. Migration of the tethered leukocyte through the intima is promoted by cell-type-specific chemoattractants secreted by endothelial cells, smooth muscle cells, and leukocytes (DiCorleto and Fox 2005). Chemokines also play a role in sustaining inflammation and are often expressed under the control of NF-κB. Chemokines of particular importance



Mechanisms of Endothelial Activation, Fig. 1 *Mechanisms of endothelial activation*. Clockwise from the *left*: Blood vessels are lined by a single-cell-thick layer of endothelial cells surrounded by extracellular matrix proteins and concentric layers of smooth muscle cells. Endothelial cells synthesize and secrete a variety of growth factors and vasoactive substances (*top*) to regulate smooth muscle cell growth, motility, and vessel tone. Endothelial cells also respond to growth factors to create angiogenic sprouts to form new blood vessels (*top right*). Endothelial cells actively regulate the hemostatic/thrombotic balance of circulating blood by modulating production of procoagulant and anticoagulant factors (*right*). Inflammatory activation of endothelial cells (*bottom*) is caused by a wide variety of factors and is implicated in numerous disease states, including atherosclerosis. Inflammatory factors such as TNF induce expression of leukocyte adhesion molecules and chemokines which recruit leukocytes to sites of inflammation, permit leukocyte rolling and firm adhesion to endothelial cells, and facilitate the extravasation of leukocytes into the subendothelial space. The accumulation of monocytes in the subendothelial space, and the activation of monocytes by oxidized lipoproteins and endothelial-derived factors, are critical events in the initiation of atherosclerosis

in atherosclerosis include IL-6, IL-8, and monocyte chemotactic protein 1 (MCP-1) (De Caterina et al. 2007).

Recent research has discovered that NF-κB signaling also regulates the formation and release of endothelial-derived microparticles. Microparticles are membrane vesicles less than 1 μm in diameter that are released from the cell in response to various stimuli. These microparticles can be generated by the same agents that induce adhesion molecule and cytokine expression (e.g., TNF and thrombin) and are functionally heterogenous (Rabelink et al. 2010). Depending on their lipid and protein compositions,

these NF-κB-regulated microparticles can be procoagulant and chemotactic or promote leukocyte adhesion to the endothelium (Rautou et al. 2011). Circulating levels of endothelial-derived microparticles increase at early stages in atherosclerosis and may contribute to disease progression, in part, by reducing eNOS phosphorylation and the bioavailability of NO (Rautou et al. 2011). The overall contribution of microparticles to vascular disease progression remains uncertain. However, as a functionally heterogenous group of bioactive substances, microparticles may represent an additional target of pathophysiologically relevant factors regulated by NF-κB transcription factors.

Altered Permeability of the Activated Endothelium

LDL, in a pathway common to many cells, normally enters endothelial cells through an LDL receptor-mediated endocytic route, leading to the hydrolysis of cholesteryl ester and reesterification of free cholesterol. This pathway is usually downregulated in hypercholesterolemic animals. Experiments have shown enhanced endothelial permeability to plasma lipoproteins in hypercholesterolemic animals, leading to plasma-derived buildup of LDL and VLDL particles in the subendothelial space (De Caterina et al. 2007). This accumulation of relatively large lipoproteins and lipids results from changes in vessel wall permeability in response to blood-borne inflammatory molecules in the local environment. Agonists such as histamine transiently open intercellular junctional proteins, allowing plasma-derived lipids and lipoproteins to become trapped in the subendothelial space (DiCorleto and Fox 2005). Once there, these lipoproteins can become oxidized by free radicals released by endothelial cells and leukocytes located within the lesion. Conversion of LDL to oxidized forms allows the particles to be recognized and taken up by various scavenger receptors, including CD36. Further, other products of lipid and lipoprotein oxidation function to induce changes in endothelial behavior. Cell culture experiments show that atherogenic concentrations of both oxidized LDL and unmodified LDL trigger the binding of monocytes to endothelial cells by activating transcription of the adhesion molecules VCAM-1 and ICAM-1 (DiCorleto and Fox 2005). Other functional changes, such as alterations in motility, and the expression of growth factors and cytokines have also been reported.

Thrombogenicity and Vasospasm

The healthy endothelium actively inhibits coagulation. Endothelial synthesis of prostacyclin and other antithrombotic factors inhibit platelet aggregation and adherence to the endothelium. During endothelial dysfunction, however, localized alterations in the balance of hemostatic factors may render the endothelial surface thrombogenic (Fig. 1, right). Endothelial expression of the pro-thrombotic tissue factor can be induced by a variety of agonists including thrombin, TNF, shear stress, and oxidized lipoproteins (Chiu and Chien 2011; DiCorleto and Fox 2005). Additionally, changes in the relative expression of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) contribute to increased thrombogenicity of the activated endothelium. Under normal conditions, the expression of these enzymes is tightly regulated to maintain a hemostatic/thrombotic balance. Activated endothelial cells tend to express higher levels of PAI-1, which can be induced by oxidized lipoproteins and shear stress, and lower levels of tPA (DiCorleto and Fox 2005). Together, these changes reduce the rate of fibrinolysis at localized sites of endothelial cell activation, creating a more thrombogenic environment.

The vasorelaxants NO and prostacyclin are key mediators of vascular tone. Availability of NO is curtailed in a number of vascular pathologies, including atherosclerosis and coronary artery disease, and is considered characteristic of vascular disease (De Caterina et al. 2007). Reductions in NO availability occur though multiple mechanisms, including decreased NO production, stabilization, or inactivation by ROS. A number of endothelial-derived vasoconstrictive substances antagonize the actions of NO and prostacyclin to induce smooth muscle cell

contraction (Fig. 1, top). ET-1 is the most potent and perhaps the most pathophysiologically important vasoconstrictor reported to date. It is expressed as a propeptide by endothelial cells and is activated by proteolytic cleavage mediated by ET-converting enzyme. Endothelial cells secrete most of the protein basolaterally, where the active form interacts with endothelin receptors on smooth muscle cells to induce vasoconstriction. Multiple atherogenic factors induce ET-1, including lipoproteins, interferon-γ, IL-1β, thrombin, and shear stress. Aberrant expression of endothelial-derived ET-1 may function in a number of vascular diseases, including atherosclerosis, where it is expressed in atherosclerotic vessels and contributes to pathophysiological vasoconstriction (Lüscher et al. 2005; De Caterina et al. 2007).

Biomechanical Forces and Endothelial Activation

As previously discussed, the location of the endothelium exposes it to a variety of biomechanical forces, which regulate cell shape, growth, and gene expression. Mechanotransduction of laminar flow and high shear stress is normally adaptive and atheroprotective, but low-level shear stress or disturbed flow is atherogenic. Atherosclerotic lesions form at sites of disturbed blood flow in arterial vessels, suggesting that changes in shear stress may alter patterns of atheroprotective or atherogenic gene expression (Chiu and Chien 2011). While the mechanisms by which disturbed blood flow contributes to lesion formation are not completely understood, some differences in the activation of signaling pathways between laminar and disturbed flow have been observed. Both types of flow activate intracellular signaling kinases and transcription factors including NF-κB, early growth response 1 (EGR1), activator protein 1 (AP1), and JUN N-terminal kinase (JNK) (Hahn and Schwartz 2009). Under laminar flow, the activation of these molecules is transient, increasing over several hours and then returning to basal levels. In contrast, endothelial cells exposed to disturbed flow show sustained activation of kinases and transcription factors, including NF-κB and JNK, which coincides with increased expression of inflammatory genes, such as ICAM-1, VCAM-1, E-selectin, MCP-1, ET-1, and PDGF (Chiu and Chien 2011; Hahn and Schwartz 2009). These differences in the kinetics of pathway activation and gene expression between laminar and disturbed flow may represent a potential basis for the nonrandom formation of atherosclerotic lesions at vessel bifurcations where blood flow is disturbed or slowed.

Growth Factor Activation of the Endothelium and Angiogenesis

Normally quiescent under physiological conditions, endothelial cells can be activated by growth factors to acquire proliferative, migratory, and invasive properties. Important stimulators of endothelial cell proliferation include fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), PDGF, and epidermal growth factor (EGF) (Manka et al. 2005). These substances also induce angiogenesis, the formation of new blood vessels from the preexisting vasculature.

The primary event governing the initiation of angiogenesis is the formation of an endothelial cell sprout from a preexisting endothelial cell monolayer (Potente et al. 2011). Environmental cues, primarily in the form of growth factors such as VEGF, cause normally quiescent endothelial cells to become activated and acquire a migratory and invasive phenotype (Herbert and Stainier 2011; Potente et al. 2011). This phenotypic change coincides with a reduction in cell-to-cell contacts and a corresponding increase in vascular permeability (Herbert and Stainier 2011). Based on relative amounts of VEGF receptor-2 (VEGFR-2), a "tip cell" (TC, high VEGFR-2 expression) is selected to lead an endothelial cell sprout to form a new vessel. The TC leads migration by following chemoattractant guidance cues and degrading extracellular matrix (ECM) proteins. Other activated endothelial cells, termed "stalk cells," (SC) follow behind TCs, maintain a proliferative phenotype, and form a rudimentary new vessel lumen (Fig. 1, top right). Together, TCs and SCs constitute the

endothelial cell sprouts that continue to migrate until an adjacent sprout is reached. Sprouts then fuse together in a process called anastomosis. Following anastomosis, the motile and proliferative phenotypes of TCs and SCs are gradually lost, and cell-to-cell junctions are reestablished to form a proper lumen and allow for blood flow. The endothelium plays an additional role in forming a mature neovasculature by synthesizing and depositing ECM proteins as well as synthesizing growth factors such as PDGF which recruit pericytes to support the new vessel (Potente et al. 2011).

Clinical Definitions of Endothelial Dysfunction

The results from many studies using in vitro systems and animal models have led to the currently accepted definition of endothelial dysfunction. An unresolved challenge in vascular research is to create a reliable method for the clinical diagnosis of endothelial dysfunction, and various plasma biomarkers have been proposed and investigated for this purpose. In particular, plasma levels of von Willebrand factor (vWF) may be useful as a prognostic factor for cardiovascular diseases. Other potential biomarkers include ET-1, soluble adhesion molecules, angiotensin II, PDGF, asymmetric dimethylarginine ADMA, and NO (Freestone et al. 2010).

Importantly, some studies have shown that NO bioavailability is reduced in early atherosclerotic lesions (Chiu and Chien 2011). Additionally, it has long been known that ADMA is an endogenous inhibitor of NO synthesis, and ADMA levels are elevated in patients with various vascular diseases including coronary artery disease, hypertension, hypercholesterolemia, and atherosclerosis (Freestone et al. 2010; Leone et al. 1992). It has therefore been suggested that ADMA may be a plasma marker for early-stage endothelial dysfunction.

More recently, endothelial-derived microparticles in the plasma have been proposed as a diagnostic indicator of endothelial dysfunction. As previously mentioned, endothelial microparticles are known to be generated by a variety of atherogenic cytokines. Additionally, increased levels of microparticles generated from the endothelium have been observed in a variety of disease states including atherosclerosis, coronary artery disease, pulmonary hypertension, and end-stage renal failure (Rautou et al. 2011).

As the body's largest homeostatic organ, the vascular endothelium forms a marvelous non-thrombogenic surface lining every vessel in the body. Its function is critical for maintaining vascular health, and its dysfunction is associated with the genesis and progression of various vascular diseases. As a dynamic biological interface, the endothelium has the capacity to sense environmental changes, synthesize vasoactive substances, interact with other cell types, regulate vascular permeability, and form collateral vessels from the preexisting vasculature. Dysregulation of these processes is present in many vascular diseases, and endothelial dysfunction often precedes atherosclerosis and associated conditions, suggesting that endothelial activation may play a causative role in serious vascular illnesses. However, while the endothelium is known to participate in the genesis and progression of vascular diseases, the specific triggering events of endothelial activation, which distinguish pathophysiological dysfunction from physiological adaptation, are still incompletely characterized. Therefore, a more thorough understanding of endothelial dysfunction may lead to descriptions of causative events underlying endothelial dysfunction for distinct disease states. Such knowledge may allow for better risk stratification of patients before major adverse events as well as the identification of novel opportunities for therapeutic intervention to prevent or treat vascular diseases.

Cross-References

- ▶ Atherosclerosis and Cytokines
- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Dendritic Cells in Atherosclerosis
- ▶ Macrophages, Oxidative Stress, and Atherosclerosis
- ▶ NF-κB
- ▶ Nitric Oxide

References

Baltimore D. NF-κB is 25. Nat Immunol. 2011;12:683–5.
De Caterina R, Massaro M, Libby P. Endothelial Functions and Dysfunctions, in Endothelial Dysfunctions in Vascular Disease (De Caterina R, Libby P, editors). Blackwell Publishing, Oxford, UK; 2007. p. 3–25. doi: 10.1002/9780470988473.
Chiu J-J, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiol Rev. 2011;91:327–87.
DiCorleto PE, Chisolm GM. Participation of the endothelium in the development of the atherosclerotic plaque. Prog Lipid Res. 1986;25:365–74.
DiCorleto PE, Fox PL. Vascular endothelium. In: Fuster V, Topol EJ, Nabel EG, editors. Atherothrombosis and coronary artery disease. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 391–9.
Freestone B, Krishnamoorthy S, Lip GYH. Assessment of endothelial dysfunction. Expert Rev Cardiovasc Ther. 2010;8:557–71.
Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. Nat Rev Mol Cell Biol. 2009;10:53–62.
Herbert SP, Stainier DYR. Molecular control of endothelial cell behaviour during blood vessel morphogenesis. Nat Rev Mol Cell Biol. 2011;12:551–64.
Kinlay S, Ganz P. Endothelial vasodilatory dysfunction: basic concepts and practical implementation. In: Caterina RD, Libby P, editors. Endothelial dysfunctions and vascular disease. Malden: Blackwell; 2007. p. 179–88.
Leone A, Moncada S, Vallance P, et al. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet. 1992;339:572–5.
Lüscher TF, Noll G, Spieker L, et al. Coronary spasm and atherothrombosis. In: Fuster V, Topol EJ, Nabel EG, editors. Atherothrombosis and coronary artery disease. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 599–622.
Manka D, Compernolle V, Carmeliet P. Molecular basis of vasculogenesis, angiogenesis, and arteriogenesis. In: Fuster V, Topol EJ, Nabel EG, editors. Atherothrombosis and coronary artery disease. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 333–46.
Oeckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF-κB signaling pathways. Nat Immunol. 2011;12:695–708.
Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol. 2007;7:803–15.
Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell. 2011;146:873–87.
Rabelink TJ, de Boer HC, van Zonneveld AJ. Endothelial activation and circulating markers of endothelial activation in kidney disease. Nat Rev Nephrol. 2010;6:404–14.
Rautou P-E, Vion A-C, Amabile N, et al. Microparticles, vascular function, and atherothrombosis. Circ Res. 2011;109:593–606.

Micro-RNA in Autoimmunity

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Synonyms

Micro-ribonucleoprotein (miRNP); Micro-RNA (miRNA, miR); RNA polymerase II (pol II); RNA-induced silencing complex (RISC); Systemic lupus erythematosus (SLE)

Definition

Micro-RNAs are small, ∼21- to 22-nucleotide (−nt) long, noncoding regulatory RNAs that contribute to the regulation of coding gene expression.

Introduction

In vivo gain- and loss-of-function studies in mouse models demonstrate without a doubt that micro-RNAs (miRNAs), alongside with coding genes, control the mammalian immune system. The regulation of miRNA expression is tightly controlled, and often the same rules and regulations that govern coding gene expression apply also to miRNAs. Similar to coding genes, altering the levels and the temporal expression of a specific miRNA clearly affects the proper development and function of the tissue where it is expressed. Therefore, it is reasonable to argue that the dysregulated control of miRNA expression would affect immune functions and development as has been well established for coding genes. Along the same line of reasoning, there is an impetus to use miRNAs as diagnostic biomarkers and to develop therapeutic agents to target miRNAs for the treatment of various diseases such as autoimmune diseases.

Micro-RNAs are small, ~21 to 22 nucleotide (nt) long, noncoding regulatory RNAs, first discovered in *Caenorhabditis elegans* (*C. elegans*) for its role in regulating the expression of coding genes. Most mammalian miRNAs are transcribed by RNA polymerase II (pol II), the same polymerase that directs the transcription of coding genes. MiRNA genes are encoded in intergenic regions, in sense or antisense orientation within introns of specific genes, or by noncoding transcripts (Pillai et al. 2007). In the nucleus, miRNAs are derived from larger precursors called primary (pri)-miRNAs that are processed into 75-nt-long precursor (pre)-miRNA hairpins by the RNAse III enzyme Drosha. The hairpins are exported into the cytoplasm by exportin 5. In the cytoplasm, Dicer, an RNAse III-like enzyme, processes the pre-miRNA hairpins to generate small RNA duplexes. One and sometimes both of the strands will be incorporated into the RNA-induced silencing complex (RISC) or micro-ribonucleoprotein (miRNP) complex containing Argonaute 2 (Ago 2), among other proteins. The RISC/miRNP complex executes miRNA functions, and miRNAs perform their functions by forming a duplex with the target gene(s) in the 3′ untranslated (UTR) region of its messenger RNA (mRNA). Depending on the degree of complementarity between the miRNA: mRNA duplex, this interaction usually leads to the downregulation of protein expression by translational repression, mRNA cleavage, or promotion of mRNA decay (Fabian et al. 2010). Interestingly, the interaction between mRNAs and miRNAs not only affects the translation of the target mRNA, but also the miRNA stability, and this reciprocal relationship depends on the extent of base pairing between the two molecules (Ameres and Fukunaga 2010).

To date, hundreds of miRNAs have been identified in species including viruses, plants, nematodes, mice, and humans, and the number is still increasing (miRBase, http://www.mirbase.org/). Most human miRNAs are conserved in the mouse, and about one-third of *C. elegans* miRNAs have vertebrate homologues, suggesting that a large fraction of miRNAs play evolutionary conserved developmental and/or functional roles.

Effects of Global Reduction of miRNA Expression

The first hint that miRNAs play an important role in the immune system came from mouse studies in which the *dicer-1* or *Ago2* gene is conditionally deleted. The ablation of *dicer* leads to a global reduction of miRNAs and other small RNAs, and it is clear that inactivation of Dicer activity, thus the expression of most if not all miRNAs, impacts the development and differentiation of T and B lymphocytes (Thai et al. 2010). Additionally, B-cell-specific deletion of *dicer* in female mice leads to the development of autoimmunity characterized by the presence of high titers serum autoreactive B-cells. It is noteworthy that not all miRNAs are equally ablated. It is also evident that not all T-cell lineage commitment programs are subjected to Dicer control. Irrespective of the cell type examined, Dicer activity is required for cell survival and proliferation, suggesting that the defects in lymphoid development may be partially due to the reduction of a group of miRNAs controlling cell survival and proliferation. Could all these phenotypes be ascribed to miRNAs, if so, which subsets of miRNAs are involved in each cell type and at which stage of lineage commitment? The answer awaits further studies.

Although Dicer and Ago2 are among the major factors involved in miRNA biogenesis and function, they are also involved in small interfering RNA (siRNA) and other small RNA biology. Therefore, the defects observed in the above mutant mice may not be solely due to the loss of miRNAs.

Lymphocyte Development

Through several seminal genetic studies, it is apparent by 2004 that individual miRNAs exert control on the immune system. First, mice reconstituted with bone marrow cells overexpressing the thymus-enriched miRNA, miR-181, whose expression is dynamically regulated during T-cell development, have a higher and lower percentage of B- and T-cells in the

periphery, respectively. MiR-181a is also over-represented in tolerized and re-tolerized $CD8^+$ T-cells (Schietinger et al. 2012). The generation and analysis of miR-181 loss-of-function mice would be valuable to provide a more physiological flavor to these observations.

Subsequently, work from two different groups demonstrates the important role of miRNAs in B-cell development (Xiao et al. 2007; Zhou et al. 2007). Loss- and gain-of-function studies show that miR-150, a mature lymphocyte-specific miRNA, regulates B-cell differentiation by controlling the transcription factor c-myb in a dose-dependent manner (Xiao et al. 2007). Therefore, miR-150 may act as a dimmer rather than an on/off switch in the modulation of B-cell development, and it achieves this delicate balance by fine-tuning the expression levels of its targets, such as c-myb, in B-cells. Although c-myb plays an important role in both T- and B-cell development, ectopic expression or deletion of miR-150 has a stronger effect on B- than T-cell development. Thus, other miR-150 targets may be more crucial to T-cell development.

The developmental programs in B- and T-cells are controlled by many transcription factors, among which some are lineage- and stage-specifically expressed and some are more ubiquitously expressed. It is not surprising that it is the case with miRNAs such as the miR-17∼92 cluster, and mature miRNAs from this cluster are broadly but differentially expressed in various mouse tissues. Mice overexpressing the miR-17-92 cluster suffer from lymphoproliferative and autoimmune manifestations with no obvious defects in B-cell development (Xiao et al. 2008). By contrast, miR-17-$92^{-/-}$ mice have a profound impairment of both fetal and adult B-cell development while their peripheral B-cell effector function appears to remain intact (Ventura et al. 2008). These results suggest that miR-17-92 overexpression and miR-17-92 deficiency affect overlapping but not identical targets. It would be interesting to determine whether miR-17-92 deficiency would alleviate autoimmunity.

Adaptive Immunity

Many miRNAs seem to participate in both hematopoiesis and immune responses, and the same mechanisms may be employed in both processes. In addition to the dysregulated B-cell development observed, miR-$150^{-/-}$ mice (discussed above) also displayed augmented steady state levels of serum immunoglobulin (Ig) A, IgG1, IgG2b, and IgM, as well as an enhanced T-dependent immune response, perhaps due to the increased c-myb levels. It has been shown that c-myb promotes lymphocyte survival by controlling the pro-survival factor Bcl2 (Taylor et al. 1996). The data suggests that miR-150 also regulates the immune response; doing so whether through c-myb or other targets remains to be determined.

One miRNA that participates in both innate and adaptive immunity is miR-155. It does not regulate lymphocyte development. MiR-155 expression is transiently induced upon activation. Dysregulated miR-155 expression results in impaired immune responses due in part to defects in germinal center (GC) formation (Rodriguez et al. 2007; Thai et al. 2007). In addition, results from many groups clearly showed that miR-155 plays an essential role in both T- and B-cell effector function (Thai et al. 2010).

One might wonder how does miR-155 regulate B- and T-cell effector functions? Does it do so by controlling the expression of a yet to be identified master regulator or by modulating different targets? Another plausible scenario would be that miR-155 controls a pathway(s) downstream of receptor activation, which might modulate genetic/epigenetic modifications in lymphoid effector genes.

Innate Immunity

The importance of miRNAs in innate immunity is also underscored by work demonstrating that miR-223, a myeloid-specific miRNA encoded in the X chromosome, negatively regulates granulocyte lineage specification and inflammatory

response (Johnnidis et al. 2008). In the absence of miR-223 (miR-223$^{-/y}$), granulocytes are hypermature, hypersensitive to stimuli and they display enhanced fungicidal activity. As a result, miR-223$^{-/y}$ mice develop spontaneous inflammatory lung pathology and exhibit an exaggerated tissue destruction following endotoxin challenge. The mutant mice display an expanded granulocytic compartment as a result of progenitor hyperproliferation. Ablation of *Mef2c*, a transcription factor that promotes progenitor proliferation and a miR-223 target predicted with the highest confidence in miR-223$^{-/y}$ mice, does not rectify the functional defects, while the progenitor proliferation and granulocyte differentiation defects are rescued. These results suggest that miR-223 is a negative regulator of progenitor proliferation, and granulocyte differentiation and activation. In addition, the data imply that miR-223 may regulate distinct targets during granulocyte development and function.

Similar to miR-155, miR-146a is induced in human monocytes after in vitro activation by LPS, suggesting that this miRNA may be involved in mammalian microbial infection. In a recent publication, the same group demonstrates that miR-146a null mice manifest an exaggerated inflammatory response to endotoxin challenge (Boldin et al. 2011). In addition, old miR-146a$^{-/-}$ mice develop spontaneous autoimmune symptoms characterized by splenomegaly, lymphadenopathy, and multi-organ inflammation that ultimately leads to premature death. MiR-146a also regulates immunological tolerance by controlling the IFN-γ signaling pathway in Treg cells (Lu et al. 2010). Thus, miR-155 and miR-146a appear to govern both the innate and adaptive immune responses to foreign and endogenous antigens. The dysregulated expression of either miRNA will lead to the breakdown of immune regulation and tolerance in mice. Because of their inducible nature, miR-155 and miR-146a would be excellent targets for the treatment of inflammatory and autoimmune diseases without interfering with normal immune responses.

Autoimmunity

Data from animal studies highlighted above clearly demonstrate that the dysregulated expression of a subset of miRNAs involved in normal lymphoid development and functions leads to some form of autoimmunity. MiR-17-92 cluster controls targets that may contribute to autoimmunity. By contrast, miR-146 and miR-223 targets appear to suppress autoimmunity. Although overexpression of miR-155 in mice does not appear to contribute to autoimmunity, its deficiency clearly alleviates inflammatory and autoimmune manifestations.

It is suggested that miR-101 might be involved in the pathogenesis of lupus-like diseases in the *sanroque* mouse; however, there is no direct genetic evidence to support this observation (Yu et al. 2007). While the observations are intriguing, the final proof implicating miR-101 in lupus pathogenesis awaits the generation of a miR-101 mutant mouse models.

The paucity of available miRNA mouse models for the study of autoimmune diseases such as systemic lupus erythematosus (SLE) is not due to a lack of interest in linking miRNAs to autoimmune diseases. Indeed, many groups have identified miRNAs that are differentially expressed in lupus patients by miRNA array assays using mostly peripheral blood mononuclear cells (PBMCs) (Thai et al. 2010). The type of miRNAs, and their putative targets, identified in these studies ranges from those associated with innate immunity to inflammation and DNA methylation. Suggestions from the data are tantalizing, yet again the final proof requires the generation and in-depth analysis of animal models for each of these miRNAs.

Micro-RNAs as Potential Drug Targets

Due to their small size and potential roles in disease processes, miRNAs have been intensively exploited as potential drug targets by both academic institutions and biopharmaceutical companies.

Several studies have demonstrated that the introduction of a small inhibitory nucleic acid

molecule, either RNA or DNA, into animal models of disease abrogates molecular pathology and, in some cases, the disease itself. To date, only three miRNAs have been effectively targeted in vivo, miR-122, miR-155, and miR-21. The injection of an anti-miRNA modified oligonucleotide (locked nucleic acids, LNA) against the liver-specific miR-122 into mice and non-human primates selectively and effectively lowers the level of this miRNA in the liver, reduces serum cholesterol, and resolves *Hepatitis C* infection, and the effects are long-lasting (Lanford et al. 2010). The US Food and Drug Administration (FDA) approves a Clinical Phase 2a trial to assess the efficacy and tolerability of the anti-miR-122 LNA (miravirsen-SPC3649) in treatment-naïve patients with chronic *Hepatitis C* infection. This represents the first miRNA-targeted drug to enter clinical trials. At this point, no data is available to indicate if miravirsen-SPC3649 is efficacious in humans. As discussed above, the dysregulated expression of miR-155 and miR-146a has been implicated in inflammation and cancer in mice. However, to date, no targeted drugs for these miRNAs have moved into in vivo animal models or clinical trials. One group though demonstrated that it is possible to inhibit miR-155 in vivo (Zhang et al. 2012). Recently, miR-21 was effectively targeted in vivo in the lupus-prone mouse model B6-*sle123* with miR-21 LNA (Garchow et al. 2011). Since miR-21 is ubiquitously and constitutively expressed, it is not clear how targeting miR-21 would ameliorate lupus-like symptoms in their study. In addition, there is no mouse or human genetic data to support the results.

In vivo miRNA replacement therapy has not been fully explored. However, one group is successful in inhibiting cancer cell proliferation, inducing tumor-specific apoptosis and providing protection from disease progression by systemically administering miR-26a into a hepatocellular carcinoma (HCC) mouse model (Kota et al. 2009). The replacement protocol described here requires the use of adeno-associated virus to express miR-26a in vivo. It might be more advantageous to develop means to introduce miRNA mimics, thus avoiding unwanted adjuvant effects inherent in adenovirus vector.

Although results in animal models proved promising, many hurdles must be overcome before anti-miRNAs or miRNA replacement therapy could be routinely used as therapeutic agents in humans: first, the mode of delivery to achieve specificity in vivo; second, the bioavailability of theses reagents; third, the potential off-target effects of these RNA molecules; and finally the cost-effectiveness of developing large-scale RNA agents.

Perspective

One can conclude without any doubt that miRNAs participate, along with coding genes, in the regulation of complex biological processes in mammalian hematopoietic/immune system. However, there is a dearth of genetic studies in human to implicate the dysregulation of miRNA expression in the pathogenesis of human diseases. To date only one study in which targeted deletion of a miRNA region faithfully recapitulates a disease state: deletion of the *LEU2/miR-15a/16-1* region results in the onset of a form of CLL manifested by a subset of patients. What accounts for the paucity of human genetic evidence to implicate miRNAs in human diseases? Is it because most miRNAs do not act as master regulators of gene expression, but rather as modifiers of gene levels? Or most miRNAs reside in more stable genomic regions that are protected from agents that induce genetic instability? Or perhaps the direction of research carried out by miRNA biologists precludes the identification of genetic evidence implicating miRNAs in human diseases? The answer is probably all of the above. While the first two issues are beyond scientists' ability to address since the nature of biology could not be modified, the miRNA biology community could focus its creativity and resources on in-depth genetic and in vivo functional studies such as the one described for the *DLEU2/miR15a/16-1* in CLL, to definitively draw a connection between miRNA expression/function and autoimmunity.

Cross-References

- ▶ Cytotoxic T Lymphocytes
- ▶ Resolution of Inflammation
- ▶ Systemic Lupus Erythematosus, Animal Models
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Ameres SL, Fukunaga R. Riding in silence: a little snowboarding, a lot of small RNAs. Silence. 2010;1(1):8.

Boldin MP, Taganov KD, Rao DS, Yang L, Zhao JL, Kalwani M, et al. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. J Exp Med. 2011;208(6):1189–201.

Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem. 2010;79:351–79.

Garchow BG, Encinas OB, Leung YT, Tsao PY, Eisenberg RA, Caricchio R, et al. Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. EMBO Mol Med. 2011;3(10):605–15.

Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, et al. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature. 2008;451(7182):1125–9.

Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell. 2009;137(6):1005–17.

Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science. 2010;327(5962):198–201.

Lu LF, Boldin MP, Chaudhry A, Lin LL, Taganov KD, Hanada T, et al. Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. Cell. 2010;142(6):914–29.

Pillai RS, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol. 2007;17(3):118–26.

Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, et al. Requirement of bic/microRNA-155 for normal immune function. Science. 2007;316(5824):608–11.

Schietinger A, Delrow JJ, Basom RS, Blattman JN, Greenberg PD. Rescued tolerant CD8 T cells are preprogrammed to reestablish the tolerant state. Science. 2012;335(6069):723–7.

Taylor D, Badiani P, Weston K. A dominant interfering Myb mutant causes apoptosis in T cells. Genes Dev. 1996;10(21):2732–44.

Thai TH, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, et al. Regulation of the germinal center response by microRNA-155. Science. 2007;316(5824):604–8.

Thai TH, Christiansen PA, Tsokos GC. Is there a link between dysregulated miRNA expression and disease? Discov Med. 2010;10(52):184–94.

Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, et al. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. Cell. 2008;132(5):875–86.

Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, Wang J, et al. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. Cell. 2007;131(1):146–59.

Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol. 2008;9(4):405–14.

Yu D, Tan AH, Hu X, Athanasopoulos V, Simpson N, Silva DG, et al. Roquin represses autoimmunity by limiting inducible T-cell co-stimulator messenger RNA. Nature. 2007;450(7167):299–303.

Zhang Y, Roccaro AM, Rombaoa C, Flores L, Obad S, Fernandes SM, et al. LNA-mediated anti-miR-155 silencing in low-grade B-cell lymphomas. Blood. 2012;120(8):1678–86.

Zhou B, Wang S, Mayr C, Bartel DP, Lodish HF. miR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. Proc Natl Acad Sci USA. 2007;104(17):7080–5.

Microscopic Polyangiitis

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Definition

A systemic inflammatory disease characterized by necrotizing small and medium vessel vasculitis.

History

Microscopic polyangiitis (MPA) was first described as a microscopic variant of polyarteritis nodosa (Wohlwill. 1923). It was classified as a small and medium vessel vasculitis under the Chapel Hill Consensus Conference (Jennette et al. 1994). In 1950, Wainwright and Davson used the term "microscopic polyarteritis" to describe this illness (Wainwright and Davson 1950).

Epidemiology

The annual incidence of MPA has been reported to be 2–17 per million in various populations (Gibelin et al. 2011). There is no gender or age predilection for MPA, but the incidence has been reported to peak in the sixth to seventh decade.

Etiology and Pathogenesis

The etiology of MPA is unknown. The disease is characterized by necrotizing inflammation of arterioles and venules with infiltration of neutrophils and lymphocytes, often with leukocytoclasis and fibrinoid necrosis. The pathogenesis of MPA is incompletely understood but has been proposed to involve the formation of antineutrophil cytoplasmic antibodies (ANCAs) (Jennette et al. 2011). The mechanisms that lead to ANCA production are unclear. ANCAs are, however, neither absolutely necessary nor sufficient to cause histologic or clinical manifestations of MPA. The initial event in MPA may involve the priming of neutrophils with cytokines such as tumor necrosis factor alpha (TNF-α) which could be triggered by environmental factors, such as infection. Neutrophil priming results in translocation of normally intracellular neutrophil granule contents to its surface. One of the proteins translocated in this way is myeloperoxidase (MPO), an enzyme that forms the antigenic target for the perinuclear ANCA (pANCA). The binding of ANCA to MPO results in neutrophil activation, degranulation, and demise by multiple pathways that include apoptosis, necrosis, and neutrophil extracellular traps. Although this has been well documented in some preclinical models, it has been inconsistently demonstrated in clinical studies. Although there appears to be an important antigen-antibody interaction in the pathogenesis of this illness, immune complexes are not observed in tissues on biopsy. Abnormal host immune responses undoubtedly participate in the onset and maintenance of vascular injury.

Clinical Features

MPA tends to involve predominantly small and medium sized blood vessels in multiple visceral organs. In one study of patients with MPA (Guillevin et al. 1999), the mean age was 56.8 years and major manifestations included renal disease (78.8 %), weight loss (72.9 %), rash (62.4 %), fever (55.3 %), mononeuritis multiplex (57.6 %), arthralgias (50.6 %), myalgias (48.2 %), hypertension (34.1 %), lung involvement (24.7 %), alveolar hemorrhage (11.8 %), and cardiac failure (17.6 %). Patients with MPA tend not to have ear, nose, and throat (ENT) involvement or lung nodules and they lack granulomatous inflammation. These features, along with the ANCA pattern and specificity (see below), help to distinguish it from granulomatous polyangiitis (Wegener's) (GPA).

Diagnosis

The diagnosis of MPA is based on a compatible clinical presentation accompanied by laboratory test results (including a positive ANCA serology) and/or imaging studies. The clinical presentation of systemic inflammation is often accompanied by concordant laboratory tests. These include a normochromic normocytic anemia, leukocytosis, thrombocytosis, low serum albumin, abnormal urinalysis (microscopic hematuria, proteinuria, and/or red blood cell casts), and elevated acute phase reactants such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

Positive ANCAs are present in 75–80 % patients with a predominant pANCA pattern on immunofluorescence and antibodies directed at myeloperoxidase (MPO) by Enzyme Linked ImmunoSorbent Assay (ELISA) testing.

Chest imaging with CT scan without radiographic contrast can be used to evaluate pulmonary involvement in the form of capillaritis/alveolitis/diffuse alveolar hemorrhage which typically shows a "ground glass appearance," In patients with multiple episodes of previous diffuse alveolar hemorrhage or capillaritis, pulmonary fibrosis in the form of "honeycombing" may be seen. Renal biopsy in indicated cases classically demonstrates a pauci-immune (relative lack of immune complexes on immunofluorescence and electron microscopy) crescentic glomerulonephritis.

Treatment

The treatment of MPA is guided by the severity of disease. The treatment paradigm for MPA has been extrapolated from treatment of related small vessel vasculitides such as GPA. The treatment is based on the classification of MPA as severe (organ-threatening or life-threatening) or non-severe. With either type, glucocorticoids form the cornerstone of treatment. The choice of a second immunosuppressive medication is driven by the severity of disease. In 1996, the French Vasculitis Study Group devised a five-factor score (FFS) (Guillevin et al. 1996) based on patients with polyarteritis nodosa, MPA, and Churg Strauss syndrome (CSS) which included proteinuria >1 g/dL, renal insufficiency (stabilized peak creatinine >1.4 mg/dL), cardiomyopathy, severe gastrointestinal manifestations, and CNS involvement. The revised FFS (Guillevin et al. 2011) based on patients with PAN, MPA, CSS, and GPA found that age >65 years, cardiac symptoms, gastrointestinal involvement, and renal insufficiency (stabilized peak creatinine >1.69 mg/dL) were associated with a high 5-year mortality. The presence of each was assigned 1 point. ENT symptoms (most commonly affecting patients with GPA and CSS) were associated with a lower risk of death, and therefore, their absence was also assigned 1 point. For patients with non-severe disease (FFS = 0), glucocorticoids alone may be sufficient as they have been shown to result in survival rates comparable to those of patients who received glucocorticoids and cyclophosphamide. For severe disease (FFS ≥ 1), the typical treatment regimen involves high-dose glucocorticoids (often intravenous followed by oral) and either oral cyclophosphamide (2 mg/kg/day) or rituximab.

In a study that compared cyclophosphamide to rituximab for remission induction in patients with new onset or relapsing GPA or MPA, rituximab was found to be non-inferior to cyclophosphamide (Stone et al. 2010). At 3–6 months, upon achievement of remission, the cyclophosphamide may be replaced with a maintenance immunosuppression medication such as methotrexate, azathioprine, or mycophenolate mofetil (Pagnoux et al. 2008) with continued taper of glucocorticoids. The choice of the maintenance immunosuppressant should be individualized based on patient preference, comorbidities, and other factors such as thiopurine methyltransferase (TPMT) genotype status for patients being considered for azathioprine. In patients who receive rituximab as induction therapy, whether to add a maintenance immunosuppression medication or continue the glucocorticoid taper alone is controversial. In patients with non-severe disease, methotrexate along with high-dose glucocorticoids can be used to induce remission provided renal insufficiency does not contraindicate the use of methotrexate. Informed decision making by the patient in terms of risk and benefit is imperative in the selection of immunosuppression and close clinical and laboratory monitoring is warranted. Plasma exchange has been shown to be effective as adjunctive therapy to conventional immunosuppression in patients with advanced renal failure in the setting of MPA (Jayne et al. 2007).

Prognosis

The FFS was also used to derive prognostic information in patients with MPA based on the

severity of illness. Based on the original FFS of 0, 1, and > =2 were associated with 5-year mortality rates of 12 %, 26 %, and 46 %, respectively. According to the revised FFS, 5-year mortality rates for scores of 0, 1, and > =2 were 9 %, 21 %, and 40 %, respectively. For 13 patients with MPA who died during the first year, older age, renal involvement, central nervous system involvement, and possibly cardiomyopathy seemed to confer the greatest risk of death. Treatment confers a good prognosis in patients with MPA. Even in patients with a FFS = 0, with a good overall 5-year survival, glucocorticoids achieved and maintained remission in only about half of the patients (40 % patients required additional immunosuppressive therapy) (Ribi et al. 2010). ANCA-associated vasculitides (GPA, MPA, CSS, and renal limited vasculitis) can result in end-stage renal disease and account for 1–2 % of patients on renal replacement therapy (Stegeman CA. 2012); however, separate data on the contribution of MPA is unavailable.

Conclusion

MPA is a systemic small and medium vessel vasculitis that merits prompt recognition and treatment. The choice of immunosuppression is guided by the severity of illness and mandates close monitoring. The introduction of newer therapeutic agents such as rituximab and the controlled duration of use of agents such as oral cyclophosphamide with change to maintenance immunosuppression after securing disease remission have made the treatment of this illness safer and more effective.

Cross-References

- ▶ Polyarteritis Nodosa
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Vasculitis: Granulomatosis with Polyangiitis (Wegener's)

References

Gibelin A, Maldini C, Mahr A. Epidemiology and etiology of Wegener granulomatosis, microscopic polyangiitis, churg-strauss syndrome and goodpasture syndrome: vasculitides with frequent lung involvement. Semin Respir Crit Care Med. 2011; 32(3):264–73.

Guillevin L, Lhote F, Gayraud M, et al. Prognostic factors in polyarteritis nodosa and Churg-Strauss syndrome. A prospective study in 342 patients. Medicine (Baltimore). 1996;75(1):17–28.

Guillevin L, Durand-Gasselin B, Cevallos R, Gayraud M, Lhote F, Callard P, et al. Microscopic polyangiitis: clinical and laboratory findings in eighty-five patients. Arthritis Rheum. 1999;42(3): 421–30.

Guillevin L, Pagnoux C, Seror R, et al. The Five-Factor Score revisited: assessment of prognoses of systemic necrotizing vasculitides based on the French Vasculitis Study Group (FVSG) cohort. Medicine (Baltimore). 2011;90(1):19–27.

Jayne DR, Gaskin G, Rasmussen N, Abramowicz D, Ferrario F, Guillevin L, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. J Am Soc Nephrol. 2007;18(7):2180–8.

Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides: the proposal of an international consensus conference. Arthritis Rheum. 1994;37: 187–92.

Jennette JC, Falk RJ, Gasim AH. Pathogenesis of antineutrophil cytoplasmic autoantibody vasculitis. Curr Opin Nephrol Hypertens. 2011;20(3):263–70.

Pagnoux C, Mahr A, Hamidou MA, Boffa JJ, Ruivard M, Ducroix JP, et al. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. N Engl J Med. 2008;359(26):2790–803.

Ribi C, Cohen P, Pagnoux C, et al. Treatment of polyarteritis nodosa and microscopic polyangiitis without poor-prognosis factors: A prospective randomized study of one hundred twenty-four patients. Arthritis Rheum. 2010;62(4):1186–97.

Stegeman CA. Microscopic polyangiitis. In: Hoffman GS, Weyand CM, Langford CA, Goronzy JJ, editors. Inflammatory diseases of blood vessels. Chichester: Wiley Blackwell; 2012. p. 227–37.

Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med. 2010;363(3):221–32.

Wainwright J, Davson J. The renal appearances in the microscopic form of periarteritis nodosa. J Pathol Bacteriol. 1950;62(2):189–96.

Wohlwill F. Über die nur mikroskopisch erkennbare Form der Periarteritis nodosa. Virchows Arch Pathol Anat Physiol. 1923;246:36.

Mixed Connective Tissue Disease (MCTD)

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Synonyms

Mixed connective tissue disease; Sharp's syndrome

Definition

MCTD is an autoimmune syndrome in which high-titer autoantibodies to the U1 small nuclear ribonucleoprotein (anti-RNP) are present along with overlapping clinical manifestations of lupus, scleroderma, and/or inflammatory myositis. Several classification criteria for MCTD have been published that perform similarly in identifying patients with MCTD (Alarcón-Segovia and Cardiel 1989). The criteria set of Alarcon-Segovia and colleagues is particularly concise and easy to apply in clinical settings (Table 1). However, it may be less likely to identify patients with primarily pulmonary manifestations as having MCTD compared to more complex schemes (Gunnarsson et al. 2012). Clinical overlap syndromes in the absence of anti-RNP antibodies exist and are distinct from MCTD. Patients with other well-defined rheumatic diseases (especially lupus and scleroderma) may have anti-RNP antibodies without having MCTD. Among lupus patients, anti-RNP is a marker of more severe disease including an increased risk of nephritis (Kirou et al. 2005). In contrast, MCTD often follows a mild and benign clinical course, though a significant risk of lung diseases emerges in some MCTD patients later in the course of their disease (Burdt et al. 1999).

Mixed Connective Tissue Disease (MCTD), Table 1 The Alarcon-Segovia classification criteria for MCTD

1. High-titer RNP+
2. Swollen hands
3. Synovitis
4. Myositis
5. Raynaud's
6. Acrosclerosis

To be classified with MCTD, patients must have criterion 1, plus at least 3 of the remaining 5 criteria

Historical Background

MCTD was first described by Gordon Sharp and colleagues, working initially at Stanford and then at the University of Missouri-Columbia in the late 1960s and early 1970s (Sharp 2002). It was the first rheumatic syndrome to be defined in part by the presence of a specific set of autoantibodies (anti-RNP).

Controversy has surrounded MCTD regarding the extent to which it was or was not distinct from lupus and other rheumatic diseases. Reports showing that, compared to lupus, MCTD has different Class II MHC disease associations, different autoantibody epitope targeting, and can be clinically distinguished by machine learning approaches have muted these concerns to some extent (Hoffman and Greidinger 2012). Concerns remain that the name "Mixed Connective Tissue Disease" may connote too general a sense of an overlap syndrome despite its acceptance as a clearly defined clinical entity (Swanton and Isenberg 2005).

Clinical Features

The most common manifestations of MCTD include Raynaud's Phenomenon, swollen hands, arthritis, sicca complaints, and esophageal disease (Table 2). Other typical manifestations include lupus-like and/or scleroderma-like skin changes, myositis, serositis, and the potential for lung disease. The manifestations of MCTD are

Mixed Connective Tissue Disease (MCTD), Table 2 Clinical manifestations present in >50 % of MCTD patients in multiple cohorts

Raynaud's Phenomenon	>85 %
Hand swelling	>75 %
Synovitis	>75 %
Dry eyes and/or mouth	>56 %
Gastroesophageal reflux	>51 %

generally consistent across geographical and ethnic boundaries (Maldonado et al. 2008).

A nationwide retrospective study in Norway estimated the point prevalence of MCTD in adults to be 3.8/100,000, with an incidence of 2.1/1,000,000, and a 3.3:1 female predominance (Gunnarsson et al. 2011). These values are similar to those observed elsewhere.

Since the initial reports by Sharp and others, MCTD has been recognized to be a generally more mild form of rheumatic disease that tends not to be associated with the most serious complications of lupus, scleroderma, or myositis. Diffuse proliferative glomerulonephritis, scleroderma renal crisis, and acute respiratory failure due to profound muscle weakness are all rare in MCTD. There is, however one exception: at least one third of MCTD patients develop serious lung disease manifestations including pulmonary hypertension, interstitial pneumonitis, or pulmonary fibrosis. Lung disease is the predominant mechanism of death attributable to MCTD, and it tends to emerge later in the evolution of the condition.

Although MCTD may be less likely to lead to major organ complications than SLE, disease activity can be substantial and impact the ability of patients to perform their activities of daily living. Most MCTD patients typically require some level of ongoing treatment. Among patients who escape the major organ complications of MCTD, some may ultimately lose their anti-RNP antibodies and enter sustained clinical remission.

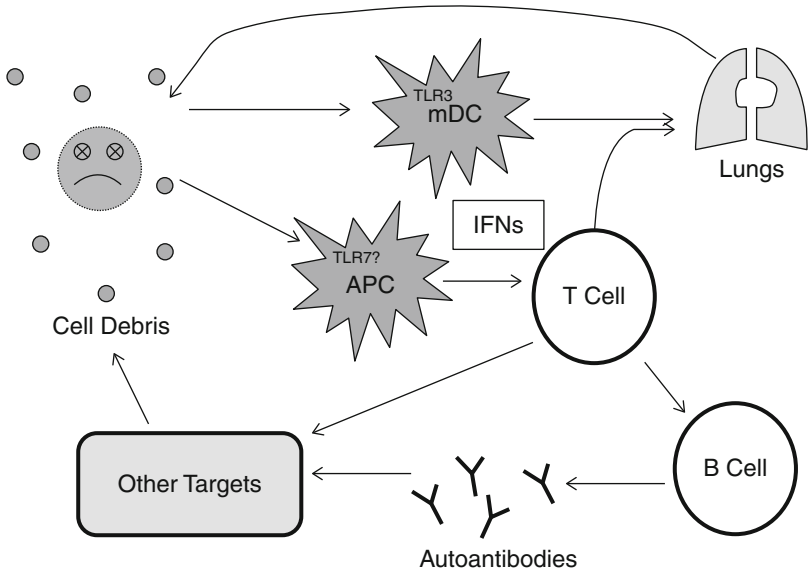
Given the potential for lung involvement in MCTD, active surveillance for evidence of lung disease with pulmonary function testing and echocardiography to assess right heart pressures is commonly performed, though these tests may miss some patients with evolving disease. Right heart catheterization to assess for pulmonary hypertension and/or high-resolution chest CT to assess for interstitial lung disease can be helpful when clinical suspicion is high. Investigators are seeking safer, less invasive tests with higher diagnostic yield to improve future disease management.

Treatment

In contrast to scleroderma lung disease in which immunosuppressive therapy has yielded minimal clinical benefit, lung disease in MCTD may be highly responsive to aggressive immunosuppression, including approximately 50 % of cases with pulmonary hypertension (Jais et al. 2008). No validated prediction rule has yet emerged to distinguish MCTD lung disease patients who will respond to immunosuppression from those who will not. The relative effectiveness of other immunosuppressive therapies (such as mycophenolate or calcineurin inhibitors) for MCTD lung disease compared to cyclophosphamide remains to be established.

There are scant treatment trials published regarding MCTD patients. The accrued evidence and anecdotal reports supports the use antimalarials, corticosteroids, disease modifyingantirheumatic drugs (DMARDs), and immunosuppressives for the treatment of MCTD activity. Treatments for organ-specific manifestations of MCTD, such as vasodilators for Raynaud's Phenomenon, proton pump inhibitors for gastroesophageal reflux disease, and topical steroids for inflammatory rashes, appear to be similarly helpful as in other rheumatic diseases.

Biologic antirheumatic therapies have not been systematically tested in MCTD. Progress in understanding the pathogenesis of MCTD may offer more opportunities to develop biologic drugs for use in this disease. Potential targets may include T cell response modifiers, anti-B cell therapies, Toll-like receptor antagonists, Type I Interferon antagonists, and IL-17 axis antagonists. Some promise exists that



Mixed Connective Tissue Disease (MCTD), Fig. 1 Putative pathogenesis of MCTD: Dead cell debris functions as a source of autoantigenic and autoinflammatory stimuli. Taken up by myeloid dendritic cells (mDC) or other antigen presenting cells (APC), the U1-RNA and other pro-inflammatory components of this cellular debris activate TLR3 and potentially TLR7 and other innate immune sensors to induce pro-inflammatory responses including elaboration of type I interferons and presentation of autoantigens to autoreactive T cells. A subset of TLR3-expressing mDC traffic to the lungs and induce the influx of autoreactivce T cells to the lungs leading to interstitial lung disease and pulmonary hypertension. Under the influence of other APC, T cells traffic to other target organs and support the development of antigen-driven autoantibody responses that also induce end organ pathology in target tissues. Target tissue injury induces additional cell debris that can cause perpetuation and amplification of the autoimmune process

antigen-targeted tolerogenic therapies may also be effective in anti-RNP autoimmunity, based on animal models and studies in SLE (Page et al. 2009; Trivedi et al. 2010).

Pathogenesis

Autoantigen-specific T cells and immunoglobulin have been identified in MCTD patients (Greidinger and Hoffman 2005). Patterns of epitope spreading in MCTD patients over time implicate immune responses against U1 snRNP macromolecules. Early recognition of apoptosis-specific RNP epitopes in some patients suggests that, as has been hypothesized in lupus, apoptotic cell debris may constitute a significant source of antigenic stimulus in MCTD. RNP antigens targeted in MCTD are also susceptible to other forms of posttranslational modification that have been hypothesized to have relevance to autoimmune disease pathogenesis, including metal-catalyzed oxidation, granzyme B cleavage, phosphorylation, and RNA conjugation (Hof et al. 2005). The RNA backbone of the U1 snRNP has been found to be a potent agonist of Toll-like receptors 3 and 7 (Greidinger et al. 2007).

A murine model of MCTD induced by immunization with an RNP peptide and U1-RNA has supported the model of MCTD as an antigen-driven immune process dependent on TLR activation (Greidinger et al. 2006). Oligoclonal anti-RNP T cell responses are seen in the mice that are homologous to those seen in human patients (Greidinger et al. 2008). Adoptive transfer studies have shown the importance of myeloid dendritic cells for the development of MCTD-like lung disease (Greidinger et al. 2009). A figure integrating the established data regarding MCTD pathogenesis (Fig. 1) must be regarded as largely speculative.

Cross-References

- Complement in Rheumatic Diseases
- Discoid Lupus
- Juvenile Diseases: SLE in Children
- Raynaud's Phenomenon
- Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- Scleroderma-Like Conditions of the Skin
- Skin in Systemic Lupus Erythematosus
- Systemic Lupus Erythematosus, Animal Models
- Systemic Lupus Erythematosus, Autoantibodies
- Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- Systemic Lupus Erythematosus, Pathogenesis
- Systemic Lupus Erythematosus, Treatment
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Alarcón-Segovia D, Cardiel MH. Comparison between 3 diagnostic criteria for mixed connective tissue disease. Study of 593 patients. J Rheumatol. 1989;16(3):328–34.

Burdt MA, Hoffman RW, Deutscher SL, Wang GS, Johnson JC, Sharp GC. Long-term outcome in mixed connective tissue disease: longitudinal clinical and serologic findings. Arthritis Rheum. 1999;42(5):899–909.

Greidinger EL, Hoffman RW. Autoantibodies in the pathogenesis of mixed connective tissue disease. Rheum Dis Clin North Am. 2005;31(3):437–50. vi.

Greidinger EL, Zang Y, Jaimes K, Hogenmiller S, Nassiri M, Bejarano P, Barber GN, Hoffman RW. A murine model of mixed connective tissue disease induced with U1 small nuclear RNP autoantigen. Arthritis Rheum. 2006;54(2):661–9.

Greidinger EL, Zang Y, Martinez L, Jaimes K, Nassiri M, Bejarano P, Barber GN, Hoffman RW. Differential tissue targeting of autoimmunity manifestations by autoantigen-associated Y RNAs. Arthritis Rheum. 2007;56(5):1589–97.

Greidinger EL, Zang YJ, Jaimes K, Martinez L, Nassiri M, Hoffman RW. CD4+ T cells target epitopes residing within the RNA-binding domain of the U1-70-kDa small nuclear ribonucleoprotein autoantigen and have restricted TCR diversity in an HLA-DR4-transgenic murine model of mixed connective tissue disease. J Immunol. 2008;180(12):8444–54.

Greidinger EL, Hoffman RW. Mixed connective tissue disease. In: Wallace DJ, Hahn BH,editors. 9th ed. Dubois' Lupus Erythematosus and Related Syndromes; Philadelphia: Elsevier, 2013.

Greidinger EL, Zang Y, Fernandez I, Berho M, Nassiri M, Martinez L, Hoffman RW. Tissue targeting of anti-RNP autoimmunity: effects of T cells and myeloid dendritic cells in a murine model. Arthritis Rheum. 2009;60(2):534–42.

Gunnarsson R, Molberg O, Gilboe IM, Gran JT. PAHNOR1 study group. The prevalence and incidence of mixed connective tissue disease: a national multicentre survey of Norwegian patients. Ann Rheum Dis. 2011;70(6):1047–51.

Gunnarsson R, Aaløkken TM, Molberg O, Lund MB, Mynarek GK, Lexberg AS, Time K, Dhainaut AS, Bertelsen LT, Palm O, Irgens K, Becker-Merok A, Nordeide JL, Johnsen V, Pedersen S, Prøven A, Garabet LS, Gran JT. Prevalence and severity of interstitial lung disease in mixed connective tissue disease: a nationwide, cross-sectional study. Ann Rheum Dis. 2012;71:1966–72. May 1. [Epub ahead of print].

Hof D, Raats JM, Pruijn GJ. Apoptotic modifications affect the autoreactivity of the U1 snRNP autoantigen. Autoimmun Rev. 2005;4(6):380–8.

Jais X, Launay D, Yaici A, Le Pavec J, Tchérakian C, Sitbon O, Simonneau G, Humbert M. Immunosuppressive therapy in lupus- and mixed connective tissue disease-associated pulmonary arterial hypertension: a retrospective analysis of twenty-three cases. Arthritis Rheum. 2008;58(2):521–31.

Kirou KA, Lee C, George S, Louca K, Peterson MG, Crow MK. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. Arthritis Rheum. 2005;52(5):1491–503.

Maldonado ME, Perez M, Pignac-Kobinger J, Marx ET, Tozman EM, Greidinger EL, Hoffman RW. Clinical and immunologic manifestations of mixed connective tissue disease in a Miami population compared to a Midwestern US Caucasian population. J Rheumatol. 2008;35(3):429–37.

Page N, Schall N, Strub JM, Quinternet M, Chaloin O, Décossas M, Cung MT, Van Dorsselaer A, Briand JP, Muller S. The spliceosomal phosphopeptide P140 controls the lupus disease by interacting with the HSC70 protein and via a mechanism mediated by gammadelta T cells. PLoS One. 2009;4(4):e5273. Epub 2009 Apr 23.

Sharp GC. MCTD: a concept which stood the test of time. Lupus. 2002;11(6):333–9.

Swanton J, Isenberg D. Mixed connective tissue disease: still crazy after all these years. Rheum Dis Clin North Am. 2005;31(3):421–36. v.

Trivedi S, Zang Y, Culpepper S, Rosenbaum E, Fernandez I, Martinez L, Hoffman RW, Greidinger EL. T cell vaccination therapy in an induced model of anti-RNP autoimmune glomerulonephritis. Clin Immunol. 2010;137(2):281–7.

Mucous Membrane Pemphigoid

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Synonym

Cicatricial pemphigoid

Definition

Mucous membrane pemphigoid (MMP), previously known as "cicatricial pemphigoid," (Chan et al. 2002) is a chronic, autoimmune, subepithelial blistering disorder characterized by involvement of predominantly mucosal surfaces and a tendency for scarring.

HLA type DQB1*0301(DQ7) is common amongst patients with both MMP and classic bullous pemphigoid.

Clinical

A chronic and progressive disease, MMP may be associated with substantive morbidity due to the tendency for scarring and fibrosis. When present on the conjunctivae, these effects may lead to blindness.

The histopathologic presentation of MMP is similar to that observed in classic bullous pemphigoid but with fewer eosinophils and more plasma cells being present in the lymphohistiocytic infiltrate that surrounds the subepidermal blisters. Mast cells and other mononuclear leukocytes are also found. Later stages also show granulation tissue in the submucosa and the characteristic fibrosis in the upper dermis.

Pathophysiology

The heterogenous nature of this condition is reflected in the variety of antigenic targets that are associated with mucous membrane pemphigoid. The principal antigens for pathogenic autoantibodies, which are all in the basement membrane zone, are the hemidesmosomal bullous pemphigoid antigen 180 (BP180, BPAG2, or type XVII collagen) and laminin 5 (laminin 332), which is a component of the anchoring filaments outside the hemidesmosomal plaque. Several other pathogenic target antigens have been suggested, which include the α_6 (predominant in oral pemphigoid) and β_4 (predominant in ocular pemphigoid) subunits of the transmembrane and hemidesmosomal $\alpha_6\beta_4$ integrins (Bhol et al. 2000, 2001; Rashid et al. 2006), the major ligand for which is laminin 5. Collagen VII and BP230 have also been implicated in some cases (Chan et al. 2002). Autoantibodies are mostly IgG1 and IgG4 isotypes, with IgA1 antibodies present in a minority of cases (Bernard et al. 1991).

Despite the similarity of target antigens with bullous pemphigoid and other bullous diseases in many cases of MMP, the specific mechanisms and reasons for the mucosal predilection, chronicity, and tendency for scarring are not well understood.

For BP180, the carboxy terminal is the immunodominant epitope in oral pemphigoid, with the shed ectodomain (LABD97) autoreactive in other forms of MMP (Calabresi et al. 2007; Kromminga et al. 2002; Leverkus et al. 2001; Schmidt et al. 2001; Schumann et al. 2000). The α_3 subunit of laminin 5 is the principal autoreactive epitope in patients with laryngeal involvement; less frequently the β_3 and/or γ_2 subunits are involved (Chan et al. 2002; Seo et al. 2001).

NC16A domain-specific T cells are present in a minority of patients, which contributes to blister formation along with released TNF-α and IFN-γ. Complement is not required (Lazarova et al. 2000). Increased expression of TGF-β1 and collagen-binding heat shock protein 47 likely contributes to conjunctival scarring in ocular disease (Black et al. 2004).

Perilesional biopsy specimens for direct IF are often difficult to obtain owing to the mucosal site, but if obtained, most patients display a fine, linear deposition along the epithelial

BMZ of IgG and/or C3. Less commonly, IgA and/or IgM may be observed (Bean et al. 1972). Circulating antibodies are observed by indirect IF in only a few cases.

References

Bean SF, et al. Cicatricial pemphigoid. Immunofluorescent studies. Arch Dermatol. 1972;106(2):195–9.
Bernard P, et al. The subclass distribution of IgG autoantibodies in cicatricial pemphigoid and epidermolysis bullosa acquisita. J Invest Dermatol. 1991;97(2):259–63.
Bhol KC, et al. The autoantibodies to alpha 6 beta 4 integrin of patients affected by ocular cicatricial pemphigoid recognize predominantly epitopes within the large cytoplasmic domain of human beta 4. J Immunol. 2000;165(5):2824–9.
Bhol KC, et al. Autoantibodies to human alpha6 integrin in patients with oral pemphigoid. J Dent Res. 2001;80(8):1711–5.
Black AP, et al. Rapid effector function of circulating NC16A-specific T cells in individuals with mucous membrane pemphigoid. Br J Dermatol. 2004;151(6):1160–4.
Calabresi V, et al. Oral pemphigoid autoantibodies preferentially target BP180 ectodomain. Clin Immunol. 2007;122(2):207–13.
Chan LS, et al. The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenic factors, medical treatment, and prognostic indicators. Arch Dermatol. 2002;138(3):370–9.
Kromminga A, et al. Cicatricial pemphigoid differs from bullous pemphigoid and pemphigoid gestationis regarding the fine specificity of autoantibodies to the BP180 NC16A domain. J Dermatol Sci. 2002;28(1):68–75.
Lazarova Z, et al. Fab fragments directed against laminin 5 induce subepidermal blisters in neonatal mice. Clin Immunol. 2000;95(1 Pt 1):26–32.
Leverkus M, et al. Cicatricial pemphigoid with circulating autoantibodies to beta4 integrin, bullous pemphigoid 180 and bullous pemphigoid 230. Br J Dermatol. 2001;145(6):998–1004.
Rashid KA, Gurcan HM, Ahmed AR. Antigen specificity in subsets of mucous membrane pemphigoid. J Invest Dermatol. 2006;126(12):2631–6.
Schmidt E, et al. Cicatricial pemphigoid: IgA and IgG autoantibodies target epitopes on both intra- and extracellular domains of bullous pemphigoid antigen 180. Br J Dermatol. 2001;145(5):778–83.
Schumann H, et al. The shed ectodomain of collagen XVII/BP180 is targeted by autoantibodies in different blistering skin diseases. Am J Pathol. 2000;156(2):685–95.
Seo SH, et al. Antiepiligrin cicatricial pemphigoid with autoantibodies to the beta subunit of laminin 5 and associated with severe laryngeal involvement necessitating tracheostomy. Dermatology. 2001;202(1):63–6.

Myositis, Pathogenesis

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Synonyms

Dermatomyositis; Inclusion body myositis; Inflammatory myopathies; Necrotizing myositis; Polymyositis

Definition

Myositis refers to a group of inflammatory myopathies (IM) that constitute a heterogeneous group of subacute, chronic, or sometimes acute acquired muscle diseases, which have in common the presence of muscle weakness and inflammation on muscle biopsy. Based on distinct clinical, histological, and immunopathological criteria, as well as different degrees of response to therapies, the most common types of myositis seen in practice can be separated into four distinct subsets: *polymyositis (PM), dermatomyositis (DM), necrotizing autoimmune myositis (NAM), and inclusion body myositis (IBM)* (Dalakas 1991, 2010a, 2011; Dalakas and Hohlfeld 2003).

PM was first recorded by Wagner in 1863 but became a recognized clinical entity 75 years later when Walton and Adams published a remarkable monograph entitled Polymyositis. The first definitive description of dermatomyositis was reported by Unverricht in 1891. Inclusion body myositis (IBM) was first recognized as a separate entity between 1967 and 1971 based on distinct

microtubular filamentous inclusions by electron microscopy in the muscle biopsies of some patients. The term "inclusion body myositis" was coined by Yunis and Samaha in 1971 to stress the uniqueness of the disease and to separate it from polymyositis. NAM has been known for some time but it has been always included within the group of PM, until the last 10 years when it has been increasingly recognized as a distinct entity attracting considerable attention for diagnosis and therapy (Dalakas 2010a, 2011).

Main Clinical Features

All forms have in common a myopathy characterized by muscle weakness which develops subacutely (weeks to months, as in PM and DM); acutely, even in days, as in some cases of NAM; or insidiously over years, as in IBM, simulating the tempo of a limb-girdle muscular dystrophy leading eventually to wheelchair confinement (Dalakas 1991, 2010a, 2011; Dalakas and Hohlfeld 2003).

DM affects both children and adults, and females more often than males, whereas PM is seen after the second decade of life and very rarely in childhood. IBM is more frequent in men than women and more likely to affect persons over the age of 50 years comprising the most commonly acquired myopathy in this age group (Dalakas 1991, 2010a, 2011; Dalakas and Hohlfeld 2003; Mastaglia et al. 2003; Engel et al. 2008); in contrast, PM is the least common while DM is the most frequent inflammatory myopathy in children.

Patients with these inflammatory myopathies have difficulty performing tasks requiring the use of proximal muscles, such as getting up from a chair, climbing steps, or lifting objects; IBM patients however may experience early difficulties with distal muscles such as inability to hold certain objects, turning keys, shaking hands, or buttoning a shirt. Falling is common among IBM patients because of early involvement of the quadriceps muscle that causes buckling of the knees and weakness of foot extensors (Dalakas 1991).

All forms of myositis can be seen with increased frequency in patients with other systemic autoimmune, viral, or connective tissue diseases. Up to 10 % of patients have interstitial lung disease and anti-Jo-1 antibodies, directed against histidyl-transfer RNA synthetase; the presence of anti-Jo-1 antibodies denotes a high probability of having or developing interstitial lung disease (Dalakas 1991, 2011; Dalakas and Hohlfeld 2003; Mastaglia et al. 2003).

Polymyositis has no unique clinical features, and its diagnosis is one of exclusion, best defined as an inflammatory myopathy of subacute onset (weeks to months) and steady progression occurring in adults that do not have the following: rash; involvement of eye and facial muscles; family history of a neuromuscular disease; endocrinopathy; history of exposure to myotoxic drugs or toxins; any neurogenic, dystrophic, or metabolic myopathy; and no signs of inclusion body myositis (Dalakas 1991, 2010a; Dalakas and Hohlfeld 2003). Unlike dermatomyositis, in which the rash secures early recognition, the actual onset of polymyositis cannot be easily determined, and the disease may exist for several months before the patient seeks medical advice.

Dermatomyositis is a distinct clinical entity identified by a rash, which accompanies or, more often, precedes the muscle weakness. The typical skin changes include a heliotrope (blue-purple discoloration) on the upper eyelids with edema; a flat red rash on the face and upper trunk, anterior chest (often in a V sign), or shoulders (shawl sign); and erythema of the knuckles with a raised violaceous scaly eruption (Gottron rash) (Dalakas 1991, 2010a, 2011; Dalakas and Hohlfeld 2003; Mastaglia et al. 2003; Engel et al. 2008).

Dermatomyositis usually occurs alone, but it may overlap with systemic sclerosis and mixed connective tissue disease. An increased incidence of malignancies is seen in patients with DM (particularly over 50 years of age), but not in PM or IBM, requiring frequent malignancy work-up and vigilance at least for the first 3 years (Dalakas 1991, 2011; Dalakas and Hohlfeld 2003). Ovarian cancer is most frequent, followed by breast, lung, and liver cancer. In Asian populations, nasopharyngeal cancer is more common.

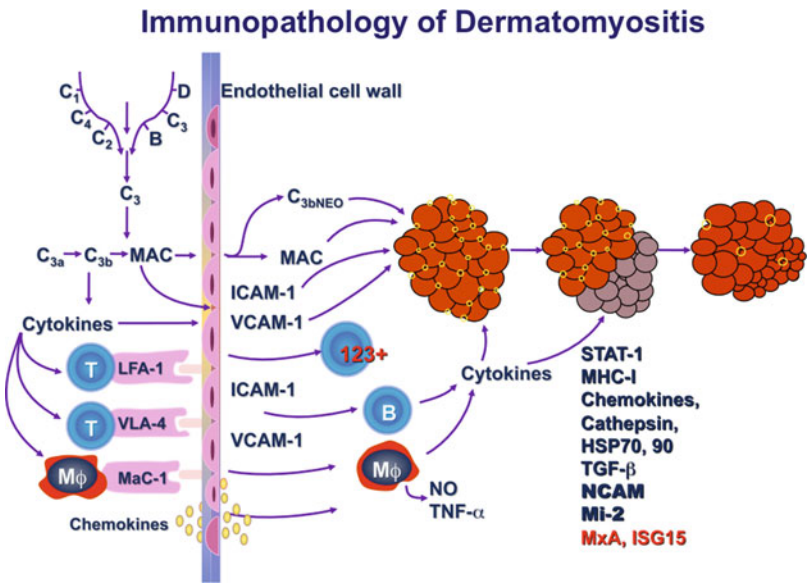
Necrotizing autoimmune myositis (NAM) has been an overlooked entity, misdiagnosed as polymyositis for many years. NAM may in fact be more common than polymyositis based on the number of cases increasingly recognized in large clinics. The patients present with very high CK, in the thousands, and moderate to severe muscle weakness of acute or subacute onset. The cause of NAM is multifactorial. Some patients have cancer or an active viral infection (i.e., HIV); others have been exposed to statins, which can induce both a toxic and an autoimmune necrotizing myositis that responds to immunotherapy; others may have a smoldering underlying autoimmune process; and still others have no other disease or apparent exposure to exogenous agents (Dalakas 2010a, 2011). The muscle biopsy shows only necrotic fibers and macrophages necessitating the absolute need to exclude dystrophic or toxic processes (Dalakas 2009), especially when the disease has subacute onset.

Inclusion body myositis has a distinct presentation with the earliest and most frequent symptom falling and tripping due to weakness and atrophy of the quadriceps muscle and foot extensors (Dalakas 1991, 2010a; Mastaglia et al. 2003). In other patients, the disease may begin with weakness of the distal muscles of the hands leading to a weak grip. Inclusion body myositis is, therefore, a proximal and distal myopathy. Weakness is asymmetrical in one third of patients (Engel et al. 2008), progresses slowly over years, and is always associated with worsening atrophy. Dysphagia and facial muscle weakness are also common.

Diagnosis

The diagnosis of DM is relatively easy when the typical skin changes are apparent. The diagnosis of PM or NAM is one of exclusion and based on finding a steadily progressive myopathy of acute or subacute onset in adults who do not have a family history of a neuromuscular disease, history of exposure to myotoxic drugs or toxins, endocrinopathy, neurogenic disease, dystrophy, or IBM (Dalakas 1991). The diagnosis of IBM should be considered in an adult who has slow-onset disease that involves distal muscles, especially foot extensors and deep finger flexors, and should be always suspected when a patient with presumed polymyositis did not respond to therapy (Dalakas 1991, 2010a; Dalakas and Hohlfeld 2003). The diagnosis is established or confirmed by elevated levels of serum muscle enzymes, electromyographic findings of an active myopathy, and, definitively, by the muscle biopsy. The serum CK, in the presence of active disease, can be elevated by as much as 50 times above normal and usually parallels disease activity; it can be however normal in active DM and IBM. In NAM, the CK is very high, usually around 8,000–15,000 IU/L.

The muscle biopsy shows distinct changes in each subset. In DM the inflammation is perivascular or at the periphery of the fascicle and is often associated with perifascicular atrophy (Dalakas 1991; Engel et al. 2008). The presence of perifascicular atrophy even in the absence of inflammation should raise the suspicion of DM. In NAM, there are necrotic fibers invaded by macrophages; T cells are characteristically absent and MHC-I is not upregulated (except in necrotic fibers). In PM and IBM, the inflammation is in multiple foci within the endomysial parenchyma and consists predominantly of CD8+ T cells that invade healthy muscle fibers expressing the MHC-I antigen which is ubiquitously upregulated on the surface of most fibers. The MHC/DC8 complex is characteristic of PM and IBM, as discussed later. In IBM, in addition to inflammation, there are rimmed vacuoles and tiny amyloid deposits in a variable number of fibers, usually in or near the vacuoles, identified with Congo-red or crystal violet stains or with Texas-red fluorescent optics. The vacuoles contain 12–16 nμ filamentous masses, reported to be identical to the paired helical filaments found in the brains of Alzheimer's disease. Ragged-red fibers with mitochondrial excess as well as cytochrome oxidase-negative fibers are frequent (Engel et al. 2008; Dalakas 2011). A number of degeneration-associated and stressor molecules accumulate within the myofibers of IBM muscles, including beta-amyloid and a series of amyloid-related proteins including phosphorylated



Myositis, Pathogenesis, Fig. 1 Immunopathogenesis of dermatomyositis. Activation of complement, possibly by autoantibodies (Y), against endothelial cells and formation of C3 via the classic or alternative pathway. Activated C3 leads to formation of C3b, C3bNEO, and membrane attack complexes (*MAC*), which are deposited in and around the endothelial cell wall of the endomysial capillaries. Deposition of MAC leads to destruction of capillaries, ischemia, or microinfarcts most prominent in the periphery of the fascicles and perifascicular atrophy. B cells, CD4 T cells, and macrophages traffic from the circulation to the muscle. Endothelial expression of vascular cell adhesion molecule (*VCAM*) and intercellular adhesion molecule (*ICAM*) is induced by cytokines released by the mononuclear cells. Integrins, specifically very late activation antigen (*VLA*)-4 and leukocyte function-associated antigen (*LFA*)-1, bind VCAM and ICAM and promote T cell and macrophage infiltration of muscle through the endothelial cell wall

tau, best detected with antibodies to transporter protein p62/SQSTMI, ubiquitin, and others (Dalakas 2010a, 2011). Currently, there is no unique molecule that serves as a specific IBM biomarker of diagnostic value.

Immunopathogenesis

Dermatomyositis

The primary antigenic target in DM is the vascular endothelium of the endomysial blood vessels (Dalakas 1991; Hohlfeld and Dornmair 2007; Engel et al. 2008). The disease begins when putative antibodies directed against endothelial cells activate complement C3 that subsequently forms C3b and C4b fragments and leads to the formation of C5b-9 (MAC), the lytic component of the complement pathway that leads to osmotic lysis of the endothelial cells and necrosis of the capillaries. This results in marked reduction in the number of capillaries per muscle fiber and dilatation of the remaining capillaries in an effort to compensate for impaired perfusion (Dalakas 1991; Engel et al. 2008). Larger intramuscular blood vessels are also affected in the same pattern, leading to muscle fiber destruction (often resembling microinfarcts) and inflammation. The perifascicular atrophy often seen in more chronic stages is a reflection of the endofascicular hypoperfusion that is prominent distally (Dalakas 2011). Membranolytic attack complex and the early complement components C3b and C4b can be detected in the serum, correlate with disease activity, and are deposited on the capillaries before inflammatory or structural changes are seen in the muscle (Engel et al. 2008; Dalakas 2010a) (Fig. 1). The endomysial infiltrates, consisting of CD4+ cells, macrophages, B cells, CD8+ cells, and plasmacytoid dendritic cells, are prominent in the perimysial, perivascular, and perifascicular regions.

The release of cytokines and chemokines related to complement activation upregulates VCAM-I and ICAM-I on the endothelial cells and facilitates the exit of lymphoid cells through the blood vessel wall to the perimysial and endomysial spaces (Fig. 1). After successful therapy, these abnormalities are no longer prominent in the repeat biopsies (Dalakas 2010a, 2011). The perifascicular regions of DM contain many regenerating or degenerating fibers and they are in a stage of continuous remodeling. As a result, they stain with alkaline phosphatase, desmin, and NCAM, with the autoantibody against chromatin remodeler Mi-2 and with a variety of antibodies against immune or stressor molecules, including TGF-β, MHC-I, αB-crystallin, cathepsins, amyloid precursor protein, STAT-1 (triggered by interferon-γ), or myxovirus-resistance MxA protein (triggered by α/β-interferon) (Dalakas 2010a, 2011). The theory that the perifascicular myofibers may be primarily injured by chronic overproduction of α/β-interferon-inducible proteins because they stain with MxA lacks specificity for DM and does not explain the aforementioned sequence of events.

Immunopathology of Polymyositis and IBM

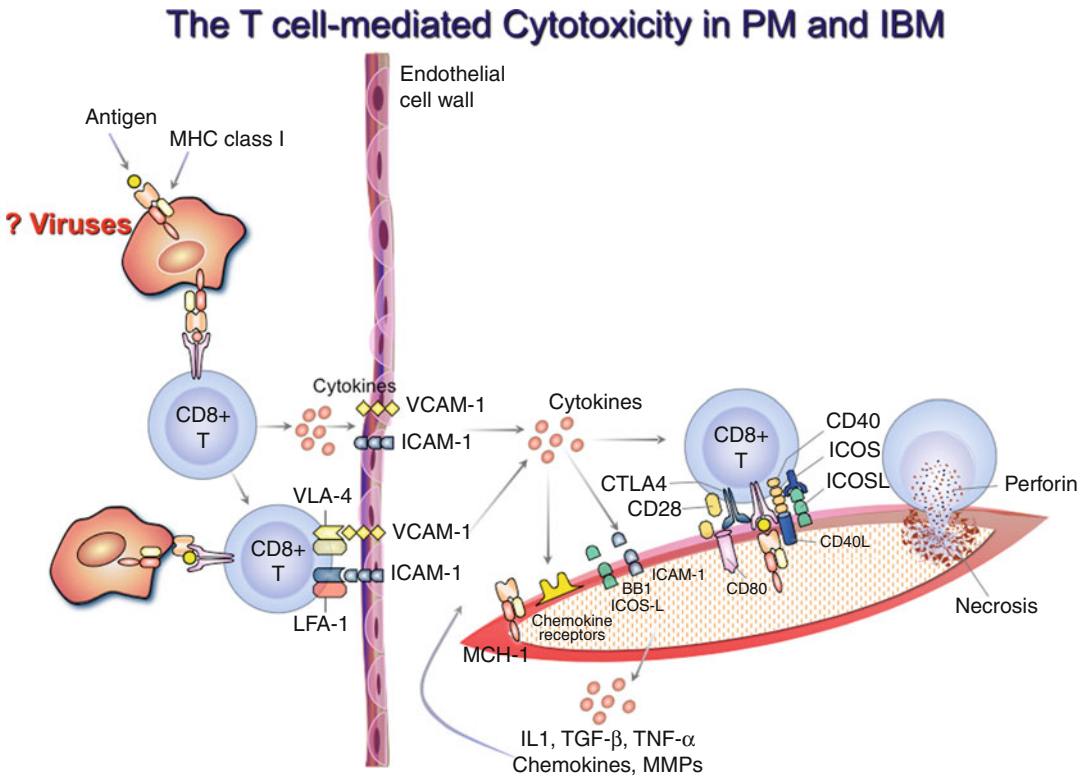
In PM and IBM, there is evidence of a T cell-mediated and MHC-1-restricted cytotoxicity process directed against heretofore unidentified muscle antigens (Engel and Arahata 1986; Emslie-Smith et al. 1989; Dalakas 1991, 2010a, 2011; Dalakas and Hohlfeld 2003; Mastaglia et al. 2003; Engel et al. 2008). The immune components associated with this process are identical in both PM and IBM, in spite of poor response to immunotherapies of the latter, and include the following (Fig. 2).

Activated, Cytotoxic CD8+ T Cells that Invade Healthy-Appearing Muscle Fibers Overexpressing MHC-I Antigen

In PM and IBM, the CD8+ T cells invading muscle fibers are activated and cytotoxic. They express ICAM-I, MHC-I, CD45RO, and inducible co-stimulator (ICOS) on their surface; send spikelike processes into nonnecrotic muscle fibers, which traverse the basal lamina and focally displace the fibers; contain perforin and granzyme granules, which are vectorially directed towards the surface of the muscle fiber inducing necrosis upon release; and are cytotoxic in vitro when exposed to autologous myotubes. An early event in PM and IBM is the widespread overexpression of MHC-I, even in areas remote from the inflammation (Engel and Arahata 1986; Emslie-Smith et al. 1989), probably induced by cytokines and chemokines such as IFN-γ or TNF-α which are found increased in the muscle fibers. The presence of perforin-positive CD8 + T cells that surround and invade MHC-I-class-expressing, nonnecrotic muscle fibers (the CD8/MHC-I lesion) is characteristic of IBM and PM and helpful to distinguish them from nonimmune myopathies (Dalakas and Hohlfeld 2003; Dalakas 2010a, 2011). In other chronic myopathies including the inflammatory dystrophies, the muscle fibers do not express the MHC-I antigen in a ubiquitous and consistent pattern as in PM and IBM, and the few T cells found in the proximity to the muscle fibers do not release cytotoxic granules.

Rearrangement of the TCR Genes of the Endomysial T Cells and Formation of Immunological Synapses Between the MHC-I-Expressing Muscle Fibers and Autoinvasive CD8+ T Cells

T cells recognize an antigen via the CDR3 region of the T cell receptors (TCR), a heterodimer of two α- and β-chains, encoded by specific genes. In patients with PM and IBM, but not in those with DM or dystrophies, only certain T cells of specific TCRα and TCRβ families are recruited to the muscle from the circulation. Cloning and sequencing of the amplified endomysial or autoinvasive TCR gene families has demonstrated a restricted use of certain gene families with conserved amino acid sequence in the CDR3 region, indicating that these cells are specifically selected and clonally expanded in situ probably driven by local antigen(s). This has been further confirmed by combining spectratyping with



Myositis, Pathogenesis, Fig. 2 Cell-mediated mechanisms of muscle damage in polymyositis (PM) and inclusion body myositis (IBM). Antigen-specific CD8 cells are expanded in the periphery, cross the endothelial barrier, and bind directly to muscle fibers via T cell receptor (TCR) molecules that recognize aberrantly expressed MHC-I. Engagement of co-stimulatory molecules (BB1 and ICOS-L) with their ligands (CD28, CTLA-4, and ICOS) along with ICAM-1/LFA-1 stabilizes the CD8–muscle fiber interaction between MHC-I and TCR (immunological synapse). Muscle fiber necrosis occurs via perforin granules released by the autoaggressive T cells. The stimulated muscle fiber also secrets myocytotoxic cytokines such as interferon (IFN)-γ, interleukin (IL)-1, or tumor necrosis factor (TNF)-α, which stimulate further the production of cytokines in an auto-amplificatory mechanism that may perpetuates disease chronicity. Death of the muscle fiber is mediated by necrosis

molecular laser-assisted microdissection and by demonstrating that the clonally expanded TCR families persist over time even in different muscles (Engel and Arahata 1986; Hofbauer et al. 2003; Salajegheh et al. 2007; Engel et al. 2008). In an important case of PM, a single clone of γ/δ T cells was the primary cytotoxic effectors that recognized genuine muscle antigens. Myeloid dendritic cells (DC), potent cells in antigen presentation, as well as large numbers of plasma cells and clonally expanded B cells are also found within the endomysial infiltrates of all inflammatory myopathies including PM, DM, and IBM (Bradshaw et al. 2007). The presence of these cells, frequently noted in the targeted tissues in several autoimmune disorders, denotes that different effector mechanisms may concurrently play an active role in the autoimmune process and contribute to muscle fiber injury.

Cytokines, Chemokines, and Adhesion Molecules

The muscle fibers and their autoinvasive CD8+ T cells coexpress co-stimulatory molecules (B7-1, B7-2, BB1, CD40, or ICOS-L) and the respective counter-receptors CD28, CTLA-4 (cytotoxic T lymphocyte antigen 4), CD40L, or ICOS (Dalakas 2011). Cytokines, chemokines,

and various adhesion and extracellular matrix molecules, such as VCAM, ICAM, thrombospondins, and metalloproteinases MMP-9 and MMP-2, are also upregulated in the tissues of patients with PM and IBM and may enhance T cell adhesion, transmigration, and cytotoxicity. Of interest, the muscle has the potential to secrete proinflammatory cytokines upon cytokine stimulation, such as INF-γ, in an auto-amplificatory mechanism that may facilitate the self-sustaining nature of endomysial inflammation and disease chronicity (Fig. 2).

Immunopathology of NAM

Although poorly studied, emerging data indicate that in NAM the main effector cell associated with muscle fiber necrosis is the macrophage. Histologically, there are a large number of necrotic fibers invaded by macrophages, but no T cell infiltrates or MHC-I expression as seen in polymyositis and inclusion body myositis (Dalakas 2010a, 2011). In a number of patients, there is deposition of complement on blood vessels (Christofer-Stine et al. 2010). It is likely that NAM is an antibody-mediated disease, as suggested by the presence of antibodies against signal recognition particles (SRP) or against a 100-kd protein corresponding to 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) (Christofer-Stine et al. 2010; Mammen et al. 2011). The recruitment of macrophages is probably consistent with an antibody-dependent cell-mediated cytotoxicity (ADCC) process (Dalakas 2010a, 2011). Because statins upregulate the expression of HMGCR in cultured cells, the major target of autoantibodies in statin-associated NAM is probably the HMGCR (Mammen et al. 2011). These autoantibodies were found in 6 % of patients with myopathies but in 92.3 % of those taking statins and aged 50 years and older.

Autoantibodies

Various autoantibodies against nuclear antigens (antinuclear antibodies) or cytoplasmic antigens are found in as many as 20 % of patients with inflammatory myopathies. These antibodies are directed against ribonucleoproteins involved in translation and protein synthesis and include various synthetases and translation factors, including Jo-1, PL-7, PL-12, OJ, EJ, PMS1, and PMS2. Of those, the antibody directed against the histidyl-transfer RNA synthetase, called anti-Jo-1, accounts for 75 % of all the antisynthetases and is clinically useful because up to 80 % of patients with Jo-1 antibodies develop interstitial lung disease. In general, the role of these antibodies in the pathogenesis of inflammatory myopathies is unclear because they are directed against ubiquitous cytoplasmic antigens with undefined function. Further, they are present in less than 15 % of the patients, and with the exception of Jo-1, they do not define an immunopathologically distinct myositis subset. Among the autoantibodies, the ones of pathogenic significance and of diagnostic value are those against SRP and HMGCR which are being evolved as specific markers of necrotizing myositis, as mentioned above.

Viral Triggers

Although several viruses have been implicated in chronic and acute myositis, sensitive PCR studies have repeatedly failed to confirm the presence of such viruses in patients' muscle biopsies. The best evidence of a viral connection is with the retroviruses. Monkeys infected with the simian immunodeficiency virus and humans infected with HIV and human T cell lymphotropic virus (HTLV-1) develop PM or IBM with typical clinical and immunopathological features as the HIV-negative myositis (Dalakas and Hohlfeld 2003; Dalakas 2010a, 2011). Viral antigens are not, however, detected within the muscle fibers but only in occasional endomysial macrophages. Molecular immunology studies using tetramers have shown that among the autoinvasive T cells, there are retrovirally specific CD8+ cells that clonally expand in situ (Dalakas et al. 2007). It is likely that the chronic infection triggers a persistent, in situ inflammatory response, which, via infected macrophages and viral-specific T cells, changes the local milieu leading to myositis.

Degenerative Pathomechanisms in IBM and Interrelationship with Inflammation

IBM is a complex disorder because in addition to the immunopathogenic events described above, there is an equally strong degenerative process as evidenced by the presence of rimmed vacuoles (almost always in fibers not invaded by T cells) and intracellular deposition of congophilic amyloid and degeneration-associated or stressor molecules, including beta-amyloid and its precursor protein, alpha-chymotrypsin, phosphorylated tau, ubiquitin, apolipoprotein E, prion protein, and others (Dalakas 2010a, 2011). One line of evidence suggests that the proteasome machinery is malfunctioning or overloaded with aberrant proteins, which may explain why these proteins accumulate in the cytoplasm of muscle fibers. The β-secretase, a major enzyme relevant for processing of amyloid precursor protein (APP), has been overexpressed in sIBM muscle fibers and may explain why processing of APP may be shifted towards the generation of β-amyloid. The aforementioned accumulations, although extensively studied in sIBM, do not seem to be unique to this disease, because they are also observed in other vacuolar myopathies. What appears unique to IBM, however, compared to other chronic vacuolar myopathies, is the concomitant accumulation of these molecules with a strong primary inflammatory response and the overexpression of proinflammatory mediators and MHC-I on all fibers, vacuolated or not. The T cell invasion appears to occur early and in higher frequency than the Congo-red-positive fibers, suggesting that inflammation precedes the accumulation of amyloid and stressor molecules. Regardless of whether the primary event in IBM is an inflammatory or protein dysregulation process, the unique coexistence of the two processes and co-localization of APP with cytokines has provided evidence that there is an interrelationship between inflammation and degeneration and that inflammatory mediators affect or increase the accumulation of degenerative molecules (Dalakas 2008, 2010a, 2011; Schmidt et al. 2008).

Therapeutic Strategies

As the specific target antigens in DM, PM, and IBM are unknown, current therapies are not selectively targeting either the autoreactive T cells or the complement-mediated process on intramuscular blood vessels. Instead, they induce nonspecific immunosuppression or immunomodulation. Furthermore, many of these therapies are empirical. Based on experience, but not controlled studies, the majority of patients with PM and DM respond to corticosteroids to some degree and for a period of time (Dalakas 2006, 2010b). Intravenous immunoglobulin (IVIg), as tested in a controlled study, is effective in DM as a second, and at times, first-line therapy (Dalakas et al. 1993). IVIg also appears to be effective in PM and NAM. Immunosuppressants are used as steroid-sparing agents but their efficacy remains unclear. New agents in the form of monoclonal antibodies or fusion proteins that target cytokines, adhesion molecules, T cell transduction or transmigration molecules, and B cells or their activation factors are emerging as promising immunotherapeutic drugs. Among them, rituximab, a B cell-depleting agent, has been helpful in some cases of DM and NAM but a controlled study – although with a problematic design – did not show efficacy (Oddis et al. 2013).

In contrast to PM and DM, there is currently no effective treatment for IBM. Prednisone, cyclosporine, azathioprine, methotrexate, total body irradiation, and IFN-β have generally failed justifying the contention that the condition could be more of a degenerative disease rather than an autoimmune condition. A number of patients with IBM however may respond to common immunotherapeutic agents early on to some degree and for a period of time; up to 25 % of patients in a controlled study have also responded transiently to IVIg. These benefits are arguably limited and short-lived; IBM remains a steadily progressive disease that, over time, is uniformly resistant to all therapies. Although transient therapeutic responses are also seen in other autoimmune diseases with poor response to immunotherapies, such as

primary progressive multiple sclerosis, the lack of treatment efficacy remains of concern and a reason for tilting the pathogenetic theories towards a primary degenerative process rather than autoimmune. It should be noted, however, that in IBM, therapy is typically initiated late when the degenerative cascade has already begun or is too advanced, due to insidious onset and very slow disease progression. This is supported by the observations that IBM patients, even with minimal clinical weakness, already exhibit significant muscle atrophy and extensive histopathologic changes.

Cross-References

- ▶ Dermatomyositis, Skin
- ▶ Juvenile Dermatomyositis
- ▶ Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Bradshaw EM, Orihuela A, McArdel SL, Salajegheh M, Amato AA, Hafler DA, Greenberg SA, O'Connor KC. A local antigen-driven humoral response is present in the inflammatory myopathies. J Immunol. 2007;178(1):547–56.

Christofer-Stine L, Casciola-Rosen LA, Hong G, Chung T, Corse AM, Mammen AL. A novel autoantibody recognizing 200 and 100 kDa proteins is associated with an immune-mediated necrotizing myopathy. Arthritis Rheum. 2010;62:2757–66.

Dalakas MC. Polymyositis, dermatomyositis, and inclusion-body myositis. N Engl J Med. 1991;325:1487–98.

Dalakas MC. Treatment of polymyositis and dermatomyositis. Signaling pathways and immunobiology of inflammatory myopathies. Nat Clin Prac Rheumatol. 2006;2:219–27.

Dalakas MC. Interplay between inflammation and degeneration: using inclusion body myositis to study neuroinflammation. Ann Neurol. 2008;64(1):1–3.

Dalakas MC. Toxic and drug-induced myopathies. J Neurol Neurosurg Psychiatry. 2009;80(8):832–8.

Dalakas MC. Inflammatory muscle diseases: a critical review on pathogenesis and therapies. Curr Opin Pharmacol. 2010a;10(3):346–52.

Dalakas MC. Immunotherapy of myositis: issues, concerns and future prospects. Nat Rev Rheumatol. 2010b;6(3):129–37.

Dalakas MC. An update on inflammatory and autoimmune myopathies. Neuropathol Appl Neurobiol. 2011;37(3):226–42.

Dalakas MC, Hohlfeld R. Polymyositis and dermatomyositis. Lancet. 2003;362:1762–3.

Dalakas MC, Illa I, Dambrosia JM, et al. A controlled trial of high-dose intravenous immunoglobulin infusions as treatment for dermatomyositis. N Engl J Med. 1993;329:1993–2000.

Dalakas MC, Rakocevic G, Shatunov A, Goldfarb L, Salajegheh M. Inclusion body myositis with human immunodeficiency virus infection: four cases with clonal expansion of viral-specific T cells. Ann Neurol. 2007;61(5):466–75.

Emslie-Smith AM, Arahata K, Engel AG. Major histocompatibility complex class-I antigen expression, immunolocalization of interferon subtypes and T-cell mediated cytotoxicity in myopathies. Hum Pathol. 1989;20:224–31.

Engel AG, Arahata K. Mononuclear cells in myopathies: quantitation of functionally distinct subsets, recognition of antigen-specific cell-mediated cytotoxicity in some diseases, and implications for the pathogenesis of the different inflammatory myopathies. Hum Pathol. 1986;17:704–21.

Engel AG, Hohlfeld R, Banker BQ. The polymyositis and dermatomyositis syndrome. In: Engel AG, Franzini-Armstrong C, editors. Myology. New York: McGraw-Hill; 2008. p. 1335–83.

Hofbauer M, Wiesener S, Babbe H, Roers A, Wekerle H, Dornmair K, Hohlfeld R, Goebels N. Clonal tracking of autoaggressive T cells in polymyositis by combining laser microdissection, single-cell PCR, and CDR3-spectratype analysis. Proc Natl Acad Sci USA. 2003;100(7):4090–5.

Hohlfeld R, Dornmair K. Revisiting the immunopathogenesis of the inflammatory myopathies. Neurology. 2007;69:1966–7.

Mammen AL, Chung T, Christopher-Stine L, Rosen P, Rosen A, Doering KR, Casciola-Rosen LA. Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme a reductase in patients with statin-associated autoimmune myopathy. Arthritis Rheum. 2011;63(3):713–21.

Mastaglia FL, Garlepp MJ, Phillips BA, Zilko PJ. Inflammatory myopathies: clinical, diagnostic and therapeutic aspects. Muscle Nerve. 2003;27:407–25.

Oddis CV, Reed AM, Aggarwal R, Rider LG, Ascherman DP, Levesque MC, Barohn RJ, Feldman BM, Harris-Love MO, Koontz DC, Fertig N, Kelley SS, Pryber SL, Miller FW, Rockette HE. RIM study group. Rituximab in the treatment of refractory adult and juvenile dermatomyositis and adult polymyositis: a randomized, placebo-phase trial. Arthritis Rheum. 2013;65(2):314–24.

Salajegheh M, Rakocevic G, Raju R, Shatunov A, Goldfarb LG, Dalakas MC. T cell receptor profiling

in muscle and blood lymphocytes in sporadic inclusion body myositis. Neurology. 2007;69(17):1672–9.
Schmidt J, Barthel K, Wrede A, Salajegheh M, Bahr M, Dalakas MC. Interrelation of inflammation and APP in sIBM: IL-1 beta induces accumulation of beta-amyloid in skeletal muscle. Brain. 2008;131(Pt 5):1228–40.

Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies

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Synonyms

DM (dermatomyositis); IBM (inclusion body myositis); Idiopathic inflammatory myopathies (IIM); Inflammatory myopathies; Myositis; PM (polymyositis)

Definition

The idiopathic inflammatory myopathies are a group of connective tissue diseases manifested by chronic inflammation of the striated muscle, characteristic cutaneous features (dermatomyositis), and a variety of systemic complications.

History

In 1886, Wagner first coined the term "polymyositis" to describe a woman presenting with muscle weakness, diffuse muscle and joint pain, swelling of the extremities, and erythema of the forearms (Oddis and Medsger 2004). In 1891, Unverricht described a 39-year-old pregnant woman with facial erythema and swollen, erythematous legs and thighs who subsequently developed myalgias with muscle weakness and atrophy. Unverricht then coined the term "dermatomyositis" to describe her condition. Inclusion body myositis was not named until more recently in 1967 when the pathology of inclusion bodies was described (Levine 2003). Criteria for the diagnosis of polymyositis and dermatomyositis were later created by Bohan and Peter in 1975 and these are still used today.

Epidemiology

The reported incidence of idiopathic inflammatory myopathy ranges from 2 to 10 new cases per million persons at risk per year (Oddis and Medsger 2004). Dermatomyositis has a bimodal incidence with a peak incidence at childhood and then again between the ages of 50 and 70. Polymyositis, in contrast, rarely occurs in childhood. The overall female to male incidence is 2.5:1, although this ratio is lower (1:1) in childhood disease and with malignancies and is much higher (10:1) when myositis occurs with a coexisting connective tissue disease. Disease onset is more frequent in the winter and spring months, especially in childhood cases of inflammatory myositis (Becezny 1935).

Classification Criteria of PM and DM

Many classification systems have been proposed for polymyositis and dermatomyositis, but the 1975 Bohan & Peter criteria are still most frequently used for defining patient groups for clinical research and to aid in diagnosing individual patients. These criteria are shown in Fig. 1. The essential elements of the criteria include proximal muscle weakness on physical examination, increased serum muscle enzymes, abnormal electromyogram (EMG), muscle biopsy consistent with myositis, and the characteristic rash of dermatomyositis. Inclusion body myositis (IBM) and amyopathic dermatomyositis (in which patients have the rash of dermatomyositis but no overt muscle weakness) are not included in the

Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies, Fig. 1 Bohan & Peter criteria for diagnosis of polymyositis and dermatomyositis. The Bohan and Peter classification criteria are shown

1. Symmetrical proximal muscle weakness
2. Muscle biopsy evidence of myositis (degeneration/regeneration, perifascicular atrophy, necrosis, phagocytosis, fiber size variation, mononuclear inflammatory infiltrate)
3. Increase in serum skeletal muscle enzymes
4. Characteristic electromyographic pattern (polyphasic, short small motor unit potentials; fibrillations; positive sharp waves; insertional irritability; high frequency repetitive discharges)
5. Typical rash of dermatomyositis (Gottron's sign, Gottron's papules, heliotrope rash)

Diagnostic Criteria:

Polymyositis:

Definite: all of 1–4

Probable: any 3 of 1–4

Possible: any 2 of 1–4

Dermatomyositis:

Definite: 5 plus any 3 of 1–4

Probable: 5 plus any 2 of 1–4

Possible: 5 plus any 1 of 1–4

Bohan and Peter classification criteria. Most experts have agreed that the Bohan and Peter criteria should be updated and such efforts are currently underway. Myositis-specific serum autoantibodies and magnetic resonance imaging (MRI) have been proposed to be added to the new criteria.

Clinical Features of PM and DM

Skeletal Muscle Weakness

The most frequent presenting symptom is insidious, progressive, painless symmetrical proximal muscle weakness that is subacute in onset occurring over weeks to months. In polymyositis and dermatomyositis, the hip flexors or pelvic girdle is often affected initially and patients often complain of difficulty walking up steps or arising from a chair. Upper extremity or shoulder girdle symptoms usually follow with patients experiencing difficulty raising their arms overhead or difficulty washing and combing their hair. Dysphagia can occur with nasal regurgitation of liquids or difficulty swallowing, indicating pharyngeal striated muscle involvement. Ocular or facial weakness is very uncommon in PM and DM and should prompt consideration of other neuromuscular diagnoses.

Skin Manifestations

Dermatomyositis has characteristic skin manifestations which may precede, develop simultaneously with, or follow muscle symptoms. Pathognomonic cutaneous features of dermatomyositis include Gottron's papules and the heliotrope rash. Gottron's papules are scaly, erythematous, or violaceous papules located over the dorsal aspect of the metacarpophalangeal and proximal and distal interphalangeal joints of the hands. Gottron's sign is a macular erythematous rash that occurs over extensor areas such as elbows, knees, and ankles (Sontheimer and Provost 2004). Sixty to eighty percent of dermatomyositis patients demonstrate either Gottron's papules or sign. The heliotrope rash is a characteristic erythematous or violaceous rash seen over the eyelids and periorbital area and is seen in fewer than 50 % of patients with dermatomyositis. A "V" sign with erythema over the anterior chest can be seen as well as the "shawl" sign with a macular erythema over the nape of the neck, upper back, and across both shoulders. The facial rash of dermatomyositis can appear similar to the malar rash of lupus but can involve the nasolabial folds and forehead which is atypical for lupus, and pruritus is a common and underrecognized complaint in dermatomyositis especially in the scalp (Shirani 2004).

Another characteristic rash is "mechanics hands" which is hyperkeratosis, scaling, and cracking/fissuring of the palms and lateral fingers, and is most frequently seen in patients with anti-synthetase myositis autoantibodies. Other common skin findings include cuticular hypertrophy, periungual erythema, nailfold telangiectasias, infarcts, and capillary dilation. These findings can be seen in both dermatomyositis and myositis in overlap with other connective tissue diseases.

Calcinosis

Soft tissue calcifications can occur in dermatomyositis, and is more common in the juvenile form of dermatomyositis (30–70 %) than in adult-onset disease (10 %) (Dalakas and Holhfeld 2003). Typically, calcinosis is associated with increased disease activity and duration. Calcinosis may be intracutaneous, subcutaneous, fascial, or intramuscular in location and is typically located at sites of compression.

Joints

Arthralgias and arthritis can occur early in the disease course. Typically, symptoms are mild; however, the arthropathy associated with anti-Jo1 antibody can be erosive and deforming.

Lung

The lung is the most common extramuscular organ affected in polymyositis and dermatomyositis and is a major cause of morbidity and mortality. Pulmonary complications of PM and DM can be due to hypoventilation from respiratory muscle weakness, aspiration pneumonias, and interstitial lung disease. Interstitial lung disease (ILD) has been reported to occur in 26–64 %, of patients with PM or DM, and can occur before, concomitantly, or after the onset of skin and muscle disease (Khan and Christopher-Stine 2011). The strongest predictors of poor outcomes in a large retrospective cohort study of PM/DM and ILD included older age, symptomatic ILD, low diffusion capacity (DLCO) and decreased forced vital capacity (FVC) at diagnosis, and usual interstitial pattern on chest computed tomography (CT) scan (Marie et al. 2011). Myositis-specific anti-synthetase autoantibodies such as anti-Jo1 were the strongest predictive marker for ILD.

Heart

Cardiac manifestations are quite common in PM and DM but are usually asymptomatic and thus under-recognized. In a long-term follow-up study of PM and DM patients, cardiovascular involvement was the most common cause of death in patients with myositis (Danko et al. 2004). Cardiac abnormalities can include arrhythmias, conduction abnormalities, cardiac arrest, congestive heart failure, myocarditis, pericarditis, angina, and fibrosis.

Gastrointestinal

Because pharyngeal muscle is striated and can also become inflamed in myositis, patients can exhibit dysphonia and swallowing dysfunction with difficulty initiating swallow and nasal regurgitation of liquids. Cricopharyngeal muscle dysfunction can also occur, resulting in dysphagia with a "blocking" or "sticking" sensation with swallowing.

Peripheral Vascular System

Raynaud's phenomenon can occur in all idiopathic inflammatory myopathies. Systemic vasculitis can occur commonly in childhood dermatomyositis, but it does not occur frequently in adults. The vascular lesions of dermatomyositis can include dermal or subcutaneous nodules, periungual infarcts, and digital ulcerations (Oddis and Medsger 2004).

Differential Diagnosis

The differential diagnosis of adult polymyositis and dermatomyositis is broad and can include numerous conditions affecting skeletal muscle. Inherited or genetic causes of muscle disease include the muscular dystrophies, metabolic myopathies, glycogen storage disorders, and mitochondrial myopathies. These can be

differentiated from acquired myopathies, because they are more slowly progressive and can involve a family history of myopathy. While the idiopathic inflammatory myopathies are considered acquired myopathies, other acquired myopathies can also present with skeletal muscle weakness including toxic (or drug-induced) myopathies, endocrinopathy (especially hypothyroidism), or myopathies triggered by infections such as viruses. History, physical examination, differences in laboratory tests, and muscle biopsies are essential in differentiating among these conditions.

Association with Malignancy

Epidemiologic studies have shown an association between dermatomyositis and polymyositis and a higher risk of cancer. National registries and population-based cohort studies of biopsy proven cases have found that the risk of malignancy is highest in patients with dermatomyositis. In a pooled analysis of all cases of dermatomyositis and polymyositis in Sweden, Denmark, and Finland, 618 cases of dermatomyositis were identified (Hill et al. 2001). Thirty-two percent of the DM cases had cancer, suggesting that patients with DM had a three-fold increased risk of developing cancer. The same study identified a lower but still statistically significant increased risk of cancer associated with polymyositis with a standardized incidence ratio (SIR) of 1.3. The overall risk of cancer is greatest in the first 3 years after the diagnosis of myositis. In dermatomyositis, the strongest associations are with ovarian, lung, pancreatic, breast, and colorectal cancers. Lung, bladder cancer, and non-Hodgkin's lymphoma are more strongly associated with polymyositis.

Diagnosis of PM and DM

Physical Examination

The examining physician should assess for objective signs of weakness and impaired muscle function through manual muscle strength testing which involves testing a patient's proximal and distal muscle groups against resistance and grading the strength on a 0–5 scale.

Serum Muscle Enzymes

Enzymes that leak from injured skeletal muscle into the serum are valuable in detecting muscle injury. The serum creatine kinase (CK) can be quite elevated in myositis (over 1,000 to several thousands) and is the most sensitive marker of ongoing muscle injury. Other enzymes that can also be elevated in decreasing order of sensitivity include aldolase, aspartate and alanine aminotransferases (AST and ALT), and lactate dehydrogenase (LDH) (Oddis and Medsger 2004).

Electromyography

Electromyography (or EMG) evaluates the electrical activity of muscle fibers and is a sensitive but nonspecific method of evaluating muscle inflammation. The electrical activity is recorded via surface electrodes during needle insertion, rest, and voluntary muscle contractions. Typical EMG findings in inflammatory myopathies include irritability of myofibrils on needle insertion and at rest (fibrillation potentials, complex repetitive discharges, positive sharp waves) and short duration, low amplitude complex (polyphasic) potentials on contraction. More than 90 % of patients with active myositis will have an abnormal EMG.

Muscle Biopsy

Muscle biopsy is the gold standard for confirmation of the diagnosis of inflammatory myopathy, and open surgical biopsy tends to provide more definitive pathology compared with percutaneous needle muscle biopsy. Degeneration and regeneration of myofibrils occurs in 90 % of cases. Chronic inflammatory cells in the perivascular and interstitial areas surrounding myofibrils are present in 80 % of cases and lymphocytic invasion of non-necrotic fibers is considered pathognomonic of polymyositis (Lundberg 2004). In dermatomyositis, B cells, CD4+ T cells, and the late component of complement (C5-C9, membrane attack complex) predominate in the perivascular area. Perifascicular myofibril

atrophy and endothelial cell hyperplasia of blood vessels are also noted. In contrast, polymyositis features cytotoxic T cell (mostly CD8+) invasion of myofibrils with sparing of the vasculature.

Muscle biopsies in inclusion body myositis can also demonstrate inflammatory infiltrate primarily of cytotoxic T cells, although inflammation is usually mild. The notable finding on muscle biopsy in inclusion body myositis is rimmed vacuoles representing abnormal filaments.

MRI

Magnetic resonance imaging (MRI) can be a useful, noninvasive tool for evaluating myositis (Scott and Kingsley 2004). The T1-weighted images provide anatomic detail, and normal tissue appears homogenously dark with a low signal, whereas fat appears bright. Inflammation is bright on both T1 and T2 images, but adding fat suppression to the T2 technique, in the form of short tau inversion recovery (STIR) sequences, improves the detection of muscle inflammation by enhancing the bright signal of inflammation and decreasing the fat signal (dark). Thus, T1-MRI demonstrates damage and chronicity of disease, while STIR-MRI demonstrates inflammatory disease activity.

Pathophysiology of PM and DM

The true pathogenesis of the inflammatory myopathies is unknown. The presence of T lymphocytes in muscle tissue in a majority of patients with myositis as well as serum autoantibodies suggests that immune mechanisms are involved in the pathogenesis, and both T and B cells may play a pathogenic role in these diseases. Mechanisms that have been proposed for the development of muscle inflammation include possible direct cytotoxic effects of infiltrating leukocytes such as T cells and macrophages on muscle cells, indirect effects of pro-inflammatory cytokines on muscle function, or microvessel involvement and disturbed circulation which could lead to reduced muscle function (Lundberg 2004).

Immunohistochemistry analysis of muscle tissue from myositis patients has revealed that lymphocytes such as CD8+ T lymphocytes in polymyositis and CD4+ T lymphocytes in dermatomyositis appear to invade muscle fibers, and different lymphocytes tend to aggregate in different areas of the muscle (Engel and Arahata 1986). Inflamed muscle also expresses MHC class I and II molecules in muscle fibers, which normally do not express these antigens. Although MHC class I molecules are expressed on most nucleated cells in the body, mature muscle fibers lose class I MHC expression during their differentiation from immature fibers. In myositis patients, MHC class I antigen expression is not only found in a majority of patients' muscle but can also be found distant from sites of inflammation (Karpati et al. 1988). The exact role of induced MHC expression on muscle fibers is not known. In terms of other possible cytotoxic mechanisms, perforins and granzymes have also been suggested as possible mechanisms of disease. Perforin-containing granules within CD8+ T cells have been found oriented toward adjacent muscle fibers (Goebels et al. 1996). It has been suggested that perforin may mediate the transfer of granzymes into the target cell, which may then activate caspases, leading to cell lysis and nuclear damage. Many of the myositis autoantigens are cleaved by granzyme B, and this process may play a role in creating proteins that are immunogenic and resulting in autoimmunity.

Several cytokines and chemokines have been detected in muscle tissue from patients with myositis, and it has been hypothesized that these cytokines may cause direct toxicity to muscle fibers or indirectly affect muscle fiber metabolism and contractility. The most frequently reported cytokines are pro-inflammatory cytokines IL-1α, IL-1β, and TNF-α (Lundberg 2004).

With respect to dermatomyositis, perifascicular atrophy is a unique feature of this subset of myositis, and some have speculated that this may provide a clue into the pathogenesis of DM. In muscle fibers of DM patients, capillary density tends to be highest in the middle of the fiber and lowest in the periphery of the fiber,

or the perifascicular region. Histopathologic analysis has also demonstrated that in early DM patients, there seems to be localization of the late components of complement (C5-C9) in the areas with fewer capillaries. There is also complement deposition seen on endothelial cells and dropout of endothelial cells in the perifascicular region. It is unclear if this complement deposition and possible injury to the vasculature precedes the infiltration of inflammatory cells, leading to muscle fiber damage. It has also been hypothesized that perifascicular atrophy may be the result of microvessel involvement and metabolic disturbance, leading to localized hypoxia in the perifascicular region.

A combination of immune-mediated mechanisms and different molecular pathways may ultimately contribute to the muscle pathology seen in polymyositis and dermatomyositis. The key pathways, however, are still unclear and require further research.

Myositis Autoantibodies

Serum autoantibodies can be found in up to 50 % of patients with polymyositis and dermatomyositis and can be useful in defining subsets of patients with certain distinguishing clinical features and unique phenotypes. The role of autoantibodies is unclear as it is not well understood whether they are involved in pathogenesis of myositis or just an epiphenomenon. The autoantibodies are typically classified into myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs).

Myositis-specific autoantibodies are specific for autoimmune disease as they are not associated with other neuromuscular diseases characterized by inflammation. The myositis-specific autoantibodies are classified into anti-synthetase antibodies directed against aminoacyl tRNA synthetase antigens and non-synthetase antibodies. The most frequent myositis-specific antibodies are those directed against aminoacyl-tRNA synthetase. There are 20 different aminoacyl-tRNA synthetases, and seven of them have been found to be autoantigens in myositis. Anti-synthetase antibodies are generally found in 16–26 % of patients with inflammatory myopathies. There are several well-known anti-synthetase antibodies (Fig. 2).

Anti-synthetase Myositis-Specific Antibodies

Anti-Jo1 antibodies are directed against histidyl-tRNA synthetase and are the most common MSAs since they are found in 20 % of patients with myositis (Khan and Christopher-Stine 2011). The clinical associations of the anti-synthetase antibodies are similar and have been collectively described as the "anti-synthetase syndrome." Classically, patients with anti-Jo1 autoantibodies and other anti-synthetase antibodies experience symptoms of myositis with recurrent fevers, nonerosive arthritis, mechanic's hands, Raynaud's phenomenon, and interstitial lung disease. The myositis and interstitial lung disease in this syndrome can be quite severe with multiple exacerbations, often requiring combination therapy with corticosteroids and immunosuppressive agents. It is not clear whether anti-Jo1 has a pathogenic role in myositis, but anti-Jo1 titers have also been correlated with disease activity. Longitudinal analysis of 11 patients showed modest correlation of anti-Jo1 titers and CK levels, myositis, lung, and joint disease activity (Stone et al. 2007). In three of these patients, anti-Jo1 antibody became negative when their disease was inactive.

Non-synthetase Myositis-Specific Antibodies

Non-synthetase MSAs are also associated with unique clinical phenotypes (Fig. 3). Anti-MI2 autoantibodies are directed against MI-2, a multiunit protein complex participating in transcription regulation at the chromosome level. Anti-MI2 antibodies are found in 10–30 % of adult dermatomyositis patients and are strongly associated with dermatomyositis. Anti-MI2 myopathy is characterized by severe skin manifestations of DM but has excellent response to immunosuppressive therapy. Anti-p155 is an autoantibody directed against a 155/140-kd doublet and has been found to be significantly associated with cancer in adult dermatomyositis. A more recent meta-analysis of six cohort studies involving 312 DM patients found

Antibody	Antigen	Frequency	Clinical Manifestations
Anti-Jo1	Histidyl-tRNA synthetase	18–20%	PM, DM + ILD
Anti-PL7	Threonyl-tRNA synthetase	≤3%	DM, PM + ILD
Anti-PL12	Alanyl-tRNA synthetase	≤3%	ILD > PM, DM
Anti-EJ	Glycyl-tRNA synthetase	≤2%	PM>DM + ILD
Anti-OJ	Isoleucine-tRNA synthetase	≤1%	ILD + DM, PM
Anti-KS	Asparaginyl-tRNA synthetase	≤1%	ILD > DM, PM
Anti-Zo	Phenylalanyl-tRNA synthetase	≤1%	ILD + DM, PM
Anti-Ha	Tyrosyl-tRNA synthetase	≤1%	ILD + DM, PM

Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies, Fig. 2 Myositis-specific autoantibodies: anti-synthetase antibodies. The myositis-specific anti-synthetase autoantibodies are shown. *PM* polymyositis, *DM* dermatomyositis, *ILD* interstitial lung disease, ≤ less than or equal to, > more often than (Adapted with permission from Khan and Christopher-Stine (2011))

Antibody	Antigen	Clinical Manifestations
Anti-SRP	Signal recognition particle	Severe, resistant necrotizing myopathy
Anti-MI2	Chromatin remodeling enzyme	DM with rash > muscle symptoms
Anti-SAE	Small ubiquitin-like modifier activating enzyme	DM with severe skin symptoms and rapidly progressive ILD
Anti-HMGCR	HMG-CoA Reductase	Necrotizing myopathy associated with statin
Anti-MDA5	MDA5	DM with rapidly progressive ILD, Cancer associated myositis
Anti-155/140	Transcriptional intermediary factor 1Y	Cancer associated myositis
Anti-140	Nuclear matrix protein (NXP-2)	Juvenile DM

Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies, Fig. 3 Myositis-specific autoantibodies: non-synthetase antibodies. The myositis-specific non-synthetase autoantibodies are shown. *DM* dermatomyositis, *ILD* interstitial lung disease, *HMGCR* HMG-CoA reductase, *MDA5* melanoma differentiation-associated gene 5, *SAE* small ubiquitin-like modifier-activating enzyme, > more often than (Adapted with permission from Khan and Christopher-Stine (2011))

pooled sensitivity of anti-P155 for cancer-associated myositis of 78 % and specificity of 89 % (Trallero-Araguas et al. 2012). This study found that patients with anti-P155 antibodies have 27-fold higher odds of having cancer-associated myositis than patients without the autoantibody.

Anti-SRP is an autoantibody directed against the signal recognition particle (SRP), a ribonucleoprotein involved in protein translocation across the endoplasmic reticulum. The prevalence of anti-SRP antibodies is estimated to be 5 % of myositis cases. Patients with anti-SRP myopathy present with a unique clinical syndrome with markedly elevated CK levels and rapidly progressive severe proximal muscle weakness that leads to significant disability. Patients often demonstrate early muscle atrophy and dysphagia is quite common in the later stages of the disease. Muscle biopsy specimens in anti-SRP myopathy are often distinct because they demonstrate necrosis with often scant or no inflammatory infiltrate. Staining also shows vigorous regeneration and deposition of the terminal components of the complement (C5b-9 or membrane attack complex) in the endomysial capillaries and the sarcolemma. The myopathy is also difficult to treat as it is poorly responsive to corticosteroid monotherapy.

An autoantibody directed against 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) has also been described and is

Antibody	Antigen	Clinical Manifestation
PM-Scl	Unidentified	PM/SSc overlap
U1RNP	U1 small RNP	MCTD, overlap syndrome
Non-U1 snRNPs	U2, U4/6, U5, U3 snRNPs	PM or DM/SSc or SSc overlap
Ku	DNA-binding proteins	Myositis/SSc/SLE overlap
Ro (SS-A) includes Ro60 and Ro52	RNA protein	Myositis often with SS or SLE, Ro52 with ILD
56 kDa	RNP particle	Myositis, often with Jo-1
KJ	Unidentified translation factor	PM, ILD, RP
Fer	Elongation factor 1α	Myositis
Mas	$tRNA^{ser}$-related antigen	Myositis, rhabdomyolysis, chronic hepatitis
MJ	Unidentified nuclear pore	Juvenile DM
hPMS1	Protein related to DNA repair	Myositis

Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies, Fig. 4 Myositis-associated autoantibodies. The myositis-associated autoantibodies are shown. *RNP* ribonucleoprotein, *snRNP* small nuclear ribonucleoprotein, *RP* Raynaud's phenomenon, *SLE* systemic lupus erythematosus, *SS* Sjogren's syndrome, *SSc* systemic sclerosis or scleroderma, > more often than (Adapted with permission from Khan and Christopher-Stine (2011))

associated with an autoimmune necrotizing myopathy and statin medication use. The initial study described 16 patients with necrotizing myopathy on muscle biopsy with serum that precipitated a 200/100 kd antibody (Christopher-Stine et al. 2010). In 12 of these patients over the age of 50, 83 % had been exposed to statin medications prior to the onset of weakness which was higher than age-matched DM and PM controls. Discontinuation of the statin medication did not lead to clinical improvement in these patients, and the myopathy responded well to immunosuppression. A subsequent study demonstrated that specifically regenerating muscle fibers from patients with the anti-HMGCR myopathy expressed high levels of HMG-CoA reductase (Mammen et al. 2011).

Myositis-Associated Autoantibodies

There are multiple myositis-associated autoantibodies (MAAs) which are found in patients with myositis but can also be found in other connective tissue diseases. Although these antibodies may not occur exclusively in patients with inflammatory myopathies, they can also have unique clinical phenotypes (Fig. 4). Anti-PM Scl is an antinucleolar antibody that identifies a subset of patients with myositis and features of systemic sclerosis. Anti-U1 RNP antibodies can occur in patients with "mixed connective tissue disease" and can have clinical findings of Raynaud's disease, systemic lupus erythematosus, polymyositis, dermatomyositis, or systemic sclerosis, or a combination of these diseases.

Inclusion Body Myositis

Inclusion body myositis (IBM) is included among the idiopathic inflammatory myopathies, but there is still debate whether IBM should be regarded as an inflammatory myopathy or as a degenerative myopathy with secondary inflammation. Symptoms of muscle weakness begin insidiously and progress slowly. IBM primarily affects individuals over the age of 50, and is more common in males. Although patients can manifest proximal muscle weakness, distal weakness is common and is often asymmetric.

Treatment of the Inflammatory Myopathies

Because of the rarity of the inflammatory myopathies, randomized controlled studies are lacking and treatment of these diseases remains empiric

based on case series. The mainstay of treatment is with daily oral corticosteroids initially dosed 1–2 mg/kg/day. Combination therapy with cytotoxic drugs is commonly prescribed to reduce corticosteroid side effects and to treat more aggressive therapy. A variety of cytotoxic drugs have been recommended including methotrexate, azathioprine, cyclosporine, mycophenylate mofetil, intravenous immunoglobulin (IVIg), rituximab, and cyclophosphamide.

The response to treatment of IBM has been considered so poor that most have recommended not treating the disease with immunosuppressive medications. Although several controlled trials have investigated the use of IVIg (Dalakas et al. 1997), these have shown variable responses. In many cases, weakness appears to progress despite immunosuppressive treatment.

Cross-References

- ▶ Cancer and Dermatomyositis
- ▶ Dermatomyositis, Skin
- ▶ Juvenile Dermatomyositis
- ▶ Mixed Connective Tissue Disease (MCTD)
- ▶ Myositis, Pathogenesis
- ▶ Raynaud's Phenomenon

References

Becezny R. Dermatomyositis. Arch Derm Syph. 1935;171:242–51.

Christopher-Stine L, et al. A novel autoantibody recognizing 200 and 100 kDa proteins is associated with an immune-mediated necrotizing myopathy. Arthritis Rheum. 2010;62(9):2757–66.

Dalakas MC, et al. Treatment of inclusion body myositis with IVIg: a double blind, placebo controlled study. Neurology. 1997;48:712–16.

Dalakas MC, Hohlfeld R. Polymyositis and dermatomyositis. Lancet. 2003;362:971–82.

Danko K, et al. Long-term survival of patients with idiopathic inflammatory myopathies according to clinical features: a longitudinal study of 162 cases. Medicine (Baltimore). 2004;83(1):35–42.

Engel AG, Arahata K. Mononuclear cells in myopathies: quantitation of functionally distinct subsets, recognition of antigen-specific cell-mediated cytotoxicity in some disease and implications for the pathogenesis of the different inflammatory myopathies. Hum Pathol. 1986;17:704–21.

Goebels N, et al. Differential expression of perforin in muscle-infiltrating t cells in polymyositis and dermatomyositis. J Clin Invest. 1996;97:2905–10.

Hill CL, et al. Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. Lancet. 2001;357:96–100.

Karpati G, et al. Expression of immunoreactive major histocompatibility complex products in human skeletal muscles. Ann Neurol. 1988;23:64–72.

Khan S, Christopher-Stine L. Polymyositis, dermatomyositis, and autoimmune necrotizing myopathy: clinical features. Rheum Dis Clin N Am. 2011;37:143–58.

Levine TD. History of dermatomyositis. Arch Neurol. 2003;60:780–2.

Lundberg IE. Etiology and pathogenesis of inflammatory muscle disease. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Rheumatology, Chapter 137. 4th ed. Philadelphia: Elsevier; 2004. p. 1433–48.

Mammen et al. Autoantibodies against 3-hydroxy-3-methylglutaryl-coenzyme A reductase in patients with statin-associated autoimmune myopathy. Arthritis Rheum. 2011;63(3)713–21.

Marie I, et al. Short-term and long-term outcomes of interstitial lung disease in polymyositis and dermatomyositis: a series of 107 patients. Arthritis Rheum. 2011;63:3439–47.

Oddis CV, Medsger TA. Clinical features, classification and epidemiology of inflammatory muscle disease. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Rheumatology. 4th ed, Chapter 137. New York: Mosby; 2004. p. 1433–48.

Scott DL, Kingsley GH. Use of imaging to assess patients with muscle disease. Curr Opin Rheumatol. 2004;16:678–83.

Shirani Z, et al. Pruritus in adult dermatomyositis. Clin Exp Derm. 2004;29:273–6.

Sontheimer RD, Provost TT. Cutaneous manifestations of rheumatic diseases. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2004.

Stone K, et al. Anti-Jo1 antibody levels correlate with disease activity in idiopathic inflammatory myopathy. Arthritis Rheum. 2007;56(9):3125–31.

Trallero-Araguas E, et al. Usefulness of anti-p155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and metal-analysis. Arthritis Rheum. 2012;64(2):523–32.

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Nephritogenic Antibodies in Systemic Lupus Erythematosus

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Synonyms

Nephrophilic antibodies; Pathogenic antibodies

Definition

Nephritogenic autoantibodies are autoantibodies present in patients with SLE and in mouse models of the disease (spontaneous or induced) that contribute to the pathogenesis of kidney disease in lupus.

Lupus Nephritis: Overview

Systemic lupus erythematosus (SLE), a systemic autoimmune disease, is characterized by the presence of a myriad of autoantibodies (autoAbs) to self-antigens. Clinically, SLE may affect most organs, including the kidneys, brain, skin, and blood cells, and its course is one of flares and remissions. Lupus nephritis (LN) is a leading cause of morbidity and mortality in SLE, and is reported in 40 % or more of SLE patients (Rovin and Stillman 2011). LN manifests as hematuria and/or proteinuria, diminished renal function, and eventually end-stage renal disease (ESRD). LN is more prevalent in non-Caucasian populations, which also have a greater propensity to advance to ESRD. It has been reported that non-Caucasian SLE patients have a 50 % or greater incidence of LN; while about 62 % of black LN patients progress to ESRD, only 19 % of Caucasians follow a similar course (Rovin and Stillman 2011). Histologically, LN has been classified by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) into six classes, based on the morphology of the lesion, the antibody deposition site within the glomerulus, the extent of the glomeruli involved, and the distinction between active and chronic lesions. Briefly, classes I and II reflect mesangial involvement; classes III and IV are focal and diffuse proliferative glomerulonephritis, respectively, with glomerular immune deposits; class V describes a membranous pathology; and class VI is irreversible glomerulosclerosis (Weening et al. 2004). Of note, over 90 % of SLE patients have histological changes in their kidneys, although a smaller fraction develop overt kidney disease (Rovin and Stillman 2011).

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The pathogenesis of LN is now thought to involve two steps. In early nephritis, immune complex deposition is seen in subendothelial and mesangial areas, while in late nephritis, immune deposits are seen in subepithelial and intramembranous areas (Tsokos 2011). One recent explanation advanced for the transformation from the early to the late stage is that of changes in DNase I enzyme activity in the kidney. Decreased DNase I activity can slow the rate of chromatin clearance and promote the release of metalloproteinases that degrade the glomerular basement membrane (GBM), allowing for the accumulation of immune complexes at that site (Rekvig et al. 2011).

The occurrence of numerous autoAbs in SLE has long been recognized; indeed, the presence of autoAbs comprises 2 of the 11 American College of Rheumatology criteria for the diagnosis (Tan et al. 1982). Nevertheless, their specific pathogenic role is far less understood. Clearly, some autoAbs are a diagnostic marker for lupus, but do not actually participate in tissue damage. In this entry, the focus will be on the role of SLE-associated antibodies in the pathogenesis of renal disease. Among the proposed mechanism of kidney damage mediated by SLE autoAbs are in situ immune complex deposition, leading to local inflammation via complement activation and by direct activation of inflammatory cells through Fc receptor engagement; binding directly to cell surfaces causing cytotoxicity; penetration into living cells; and binding to cross-reactive kidney antigens (Putterman 2004; Rekvig et al. 2011). While multiple autoAbs have been implicated in the pathogenesis of LN, discussed below are relatively well-established examples of nephritogenic antibodies, embodying the different types of mechanisms thought to be involved in the nephritic process.

Anti-Double-Stranded DNA Antibodies

Approximately 70 % of SLE patients are positive for anti-double-stranded DNA antibodies (anti-dsDNA Abs) which are relatively lupus specific (97 %), rendering them useful in diagnosis. Over the last few years, it has become clear that anti-dsDNA Abs have important systemic effects related to the engagement of toll-like receptor 9 (TLR 9) in dendritic cells (DCs) following internalization of dsDNA–anti-dsDNA Ab complexes. In turn, the DC become activated and secrete B-cell-activating factor (BAFF) and other pro-inflammatory cytokines, including interferon-α (IFNα). Overexpression of IFNα-inducible genes characterizes SLE patients, and IFNα expression has been shown to correlate with disease activity. IFNα overexpression then leads to a vicious cycle in which IFNα induces monocytes to differentiate into DC that themselves become activated. The activated DC then prime naive T cells to promote activation and expansion of autoreactive T and B cells, while at the same time releasing BAFF due to the TLR9 activation. In turn, more IFNα is secreted, and autoreactivity is perpetuated (Aranow et al. 2011). An analogous mechanism is thought to be relevant to RNA-binding Abs and TLR7.

Currently, there are three popular methods utilized by clinical laboratories to measure anti-dsDNA Abs levels. The Farr assay is based on the precipitation of DNA–anti DNA antibody complexes in high salt concentrations. It detects high-avidity anti-dsDNA Abs, and antibody levels measured by this method closely correlate with SLE flares, particularly nephritic ones. The sensitivity and specificity of the Farr assay for measuring anti-dsDNA Abs levels in the diagnosis of SLE are 42–85 % and 95–99 %, respectively. The Farr assay, however, is not routinely performed, as it requires the use of a radiolabeled antigen. The *Crithidia luciliae* assay is an indirect immunofluorescence assay based on the fact that the kinetoplast of this particular hemoflagellate is rich in dsDNA. *Crithidia* detects medium- to high-avidity antibodies, and shows somewhat improved specificity (98–100 %), while maintaining good sensitivity (47–55 %). Quantitation of antibody levels using *Crithidia* requires serum titration, and so it is not routinely employed to serially measure Abs levels over the course of the disease. The third, and most widely used method, is enzyme-linked immunosorbent assay (ELISA).

In this technique, serum anti-dsDNA Abs adhere to dsDNA that is fixed to a plastic well, and then detected using a secondary anti-human IgG antibody conjugated to a detector enzyme. The ELISA detects both high- and low-avidity anti-dsDNA Abs, and so is the most sensitive (56–67 %), but not as specific (91–96 %) as the two previously described methods. ELISA is technically relatively easy and usually automated. However, changes in Abs levels detected by ELISA do not always reliably reflect or predict fluctuations in disease activity (Ghirardello et al. 2007). Another relatively new, and increasingly used, method for measuring anti-dsDNA Abs (and other autoantibodies) is the multiplexed immunoassay which utilizes magnetic beads, allowing for the quantitative detection of over 10 antibodies simultaneously. This method is clearly efficient and time conserving, but its relative reliability remains to be determined.

Anti-dsDNA Abs titers have been found to correlate with SLE disease activity, and in particular with LN activity (Aranow et al. 2011). Support for the pathogenic role of anti-dsDNA Abs in LN comes from the finding of a relative enrichment of anti-dsDNA Abs in IgG eluted from LN patients' kidneys. In addition, both ex vivo and in vivo experiments in murine models have shown that anti-dsDNA Abs can promote LN: For example, perfusion of a rat kidney with monoclonal mouse and polyclonal human IgG anti-dsDNA Abs leads to proteinuria and decreased renal function, and injection of specific subsets of anti-dsDNA Abs into non-autoimmune mice produces nephritis (Putterman 2004).

While over two-thirds of SLE patients harbor anti-dsDNA Abs, many do not develop nephritis. Consequently, it was assumed, and later experimentally demonstrated in murine models, that not all of these Abs are pathogenic. It is the high-avidity, somatically mutated, cationic subsets that are particularly nephritogenic (Aranow et al. 2011). The differential pathogenic potential of specific subsets of anti-dsDNA Abs led to the following, not mutually exclusive, hypotheses as to the mechanism of anti-dsDNA Abs-mediated nephritis in SLE: the "planted" antigen hypothesis, and the cross-reactivity theory.

"Planted" Antigen

The concept of preformed circulating immune complexes that become sequestered in the glomeruli promoting an immune reaction is not currently considered valid (Aranow et al. 2011). Rather it is thought that LN is triggered by in situ formation of immune complexes, generated by recognition of exposed chromatin on the surface of apoptotic glomerular cells by circulating anti-dsDNA Abs. The "planted" immune complex then promotes mesangial proliferation through Fcγ receptors on the mesangial cells, and local activation of the immune system (Rekvig et al. 2011). Support for this theory comes from electron microscopy evidence of immune complex deposition in the GBM, and the specific recognition of GBM nucleosomes by the nephritogenic antibodies (Kalaaji et al. 2006).

Cross-Reactivity with Renal Antigen

It is important to note, however, that there are SLE patients with biopsy-proven LN, without detectable levels of anti-dsDNA Abs (Hanrotel-Saliou et al. 2011). Moreover, monoclonal anti-dsDNA Abs and human SLE serum can bind glomeruli even after eliminating nuclear material. Therefore, another hypothesis is that anti-dsDNA Abs cross-react directly with glomerular antigens, leading to local complement fixation and activation of inflammatory pathways. Accordingly, a common feature reported in many nephritogenic anti-dsDNA Abs is their ability to cross-react with an array of cell-surface and matrix components, including among others α-actinin, annexin II, laminin, and fibronectin. This hypothesis is especially pertinent as analysis of kidney-eluted IgG revealed that only about 10 % of the total IgG were Abs binding to dsDNA (Hanrotel-Saliou et al. 2011).

Since injection of some anti-dsDNA Abs to mice fails to induce glomerular injury, it is assumed for such Abs that a separate event is necessary to incite the inflammatory process and cause initial apoptosis of the glomerular cells in order to expose the chromatin to the environment. These two proposed mechanisms, DNA-mediated and cross-reactivity with renal

antigens, may then possibly work sequentially in order to trigger and amplify glomerulonephritis. In other words, circulating anti-dsDNA Abs may cross-react with resident glomerular antigens causing local inflammation, bringing about cellular apoptosis which exposes chromatin that is itself recognized by anti-dsDNA Abs – thus further intensifying the inflammatory process (Krishnan et al. 2012). Support for this sequential concept can be found in the report that anti-glomerular Abs are detected earlier than anti-nuclear Abs in kidneys of lupus-prone mice (Hanrotel-Saliou et al. 2011).

Anti α-Actinin Antibodies

α-actinin belongs to a family of actin-binding proteins, and has been described in four forms in humans: 1 and 4 are the non-muscle forms, and 2 and 3 are striated muscle forms. In the kidney, α-actinin 1, 2, and 4 are expressed by podocytes and mesangial cells. α-actinin 4 is crucial for normal kidney physiology, as α-actinin-4-deficient mice present with severe glomerular disease, while humans with point mutations in the α-actinin 4 actin-binding site develop familial focal and segmental glomerulosclerosis (Renaudineau et al. 2007).

Anti-dsDNA Abs cross-reacting with α-actinin are increased in patients with LN compared with SLE patients without LN, and track with lupus nephritis activity. Furthermore, the presence of serum α-actinin Abs is associated with a 2.5-fold increase in prevalence of LN (Youinou and Putterman 2009). In a longitudinal follow-up of LN patients, α-actinin Abs could be detected before evidence of renal disease, and the titer of the autoAbs dropped significantly following the initialization of treatment. This close association between LN activity and anti-dsDNA/α-actinin Abs implies that the cross-reactivity with α-actinin is what enables the anti-dsDNA Abs to become pathogenic (Renaudineau et al. 2007).

In concert with the human data, in murine experimental models, anti-dsDNA Abs that bound α-actinin were found to be pathogenic, while closely related anti-dsDNA Abs that differed only with regard to cross-reactivity with α-actinin were not pathogenic (Mostoslavsky et al. 2001; Deocharan et al. 2002). Moreover, polymorphisms in the *ACTN* gene were found in lupus-prone mice, leading to differential α-actinin expression (Youinou and Putterman 2009). Increased α-actinin expression may contribute to renal susceptibility to the anti-dsDNA/α-actinin Abs-mediated nephritis, as greater expression of antigen may lead to enhanced glomerular antibody deposition (Zhao et al. 2005).

Anti-annexin II Antibodies

Annexin II is a calcium-dependent, phospholipid-binding protein that is expressed on the surface of a number of phagocytic cells, including mesangial cells. A recent study identified annexin II as a human mesangial cell membrane antigen that binds anti-dsDNA Abs. It was also shown that IgG and C3 deposits in LN co-localize with annexin II in the glomeruli, and that annexin II-binding activity of IgG in the sera of SLE patients correlated both with anti-dsDNA Abs levels and nephritis activity (Yung et al. 2010). Cross-reactivity of anti-dsDNA Abs with mesangial cell annexin II may lead to antibody internalization, mesangial cell activation via the ERK intracellular signaling pathway, proliferation, and eventually apoptosis. At the same time, this process induces IL-6 as well. Thus, the presence of anti-annexin II antibodies is associated with active LN (Yung et al. 2010).

Anti-C1q Antibodies

C1q is the first component of the classical pathway of complement, binding to the Fc portion of immune complexes and initializing complement activation. C1q is important in self-antigen clearance following apoptosis as well. Structurally, it contains six globular heads responsible for Fc receptor recognition, and a collagen-like region. AutoAbs to C1q are mainly of the IgG2 isotype, and recognize epitopes in the collagen-like region. Anti-C1q antibodies were identified in SLE patients, but also in a number of other conditions, including hypocomplementemic urticarial vasculitis (HUS), Sjögren's syndrome, IgA nephropathy,

and even in normal individuals. While anti-C1q Abs are highly sensitive for HUS (found in 100 % of patients), they are neither sensitive, nor specific to SLE. However, they have been found to be more prevalent in SLE patients with LN (including both glomeruloproliferative and membranous forms), compared to patients without nephritis. In a group of 43 SLE patients followed longitudinally by Coremans et al., anti-C1q Abs were positive in 82 % of LN patients, while only 38 % of non-renal SLE patients were positive for these Abs. Furthermore, in most LN patients positive for C1q Abs, Abs titers rose significantly prior to a renal flare. At the same time, of the relatively small percentage of non-LN SLE patients who had C1q Abs, only 50 % demonstrated an increase in Abs titers prior to a non-renal flare. Of note, anti-dsDNA Abs measured in these patients over the same period of time were positive in most patients regardless of their LN status, and often rose prior to the flare, whether renal or not. This finding may indicate that fluctuations in anti-C1q Abs are more specific than changes in anti-dsDNA Abs titers for renal involvement (Coremans et al. 1995; Kallenberg 2008).

Support for a pathogenic role for anti-C1q Abs can be found in the 50-fold enrichment of anti-C1q Abs present in glomeruli of LN kidneys. It was also demonstrated that anti-C1q Abs deposit along glomerular and tubular basement membrane, in a similar pattern to C1q deposits (Mannik and Wener 1997). Nevertheless, HUS patients and some SLE patients do not develop nephritis despite having high titers of anti-C1q Abs (Sinico et al. 2009).

In experimental murine models, injection of anti-C1q Abs into normal mice led to C1q depletion, and deposition of C1q and anti-C1q Abs along the GBM. Nevertheless, only a mild granulocyte influx was noted, and no proteinuria resulted. Rather, co-treatment with anti-GBM Abs and anti-C1q Abs was required in order to bring about full-blown nephritis (Trouw et al. 2004; Kallenberg 2008). Thus, it would appear that the association of anti-C1q Abs with nephritis is through the binding of C1q to immune complexes along the GBM, and recognition of these bound C1q molecules by anti-C1q Abs. Consequently, the normal functions of C1q in immune complex solubilization and clearance of apoptotic material are disturbed, both enhancing local inflammation (Sinico et al. 2009).

Anti-ribosomal P Abs

Anti-ribosomal P Abs are detected in 12–16 % of SLE patients (Hirohata 2011), more often in Asian patients than in Caucasians, African Americans, or Hispanics (Toubi and Shoenfeld 2007). They ligate three ribosomal serine phosphorylated proteins (P0, P1, and P2) involved in the elongation step of protein synthesis when associated with the 60S subunit of the ribosome. These ribosomal proteins appear on membranes of multiple cells, including mesangial cells and blood cells (Toubi and Shoenfeld 2007).

The clinical association of anti-ribosomal P Abs with LN was demonstrated in a series of studies by Reichlin's group. In 69 SLE patients, anti-ribosomal P Abs were more prevalent in LN patients (75 %) as compared to the overall prevalence of these autoAbs in the study cohort (30 %). A strong association between anti-ribosomal P Abs and anti-dsDNA Abs was demonstrated, as 81 % of patients with positive anti-ribosomal P Abs were also positive for anti-dsDNA Abs, while only 41 % of patients without anti-ribosomal P Abs had increased titers of anti-dsDNA Abs (Chindalore et al. 1998). Additionally, in the presence of anti-ribosomal P Abs, anti-dsDNA titers were much higher in LN patients than in non-LN patients (Reichlin and Wolfson-Reichlin 2003). This can indicate cross-reactivity between the two autoAbs, although such cross-reactivity was never unequivocally proven (Hirohata 2011). Interestingly, it has been suggested that isolated anti-ribosomal P Abs are more prevalent in patients with pure membranous LN (71 % of studied patients with isolated anti-ribosomal P Abs had pure membranous LN), while the presence of anti-dsDNA Abs together with anti-ribosomal P Abs was more typically found in patients with both proliferative and membranous lesions (67 %) (Toubi and Shoenfeld 2007; Hirohata 2011).

The mechanism of pathogenicity of anti-ribosomal P Abs has not been confirmed, but a number of possibilities have been proposed. The first scenario stipulates that anti-dsDNA Abs cross-react with ribosomal P proteins. Supporting this hypothesis is the finding that the carboxyl-terminal 20 amino acids of the ribosomal P proteins were the target antigen of anti-DNA Abs on rat glomerular mesangial cells, leading to inhibited proliferation and apoptosis of these cells. Further support comes from the observations of strong association between the titers of the two autoAbs. A second possible theory relies on the fact that diffuse proliferative LN patients are skewed toward a TH1 immunologic phenotype. Anti-ribosomal P Abs (through ligation of ribosomal P epitopes on the surface of monocytes) enhance expression of IL-12, which in turn induces differentiation of CD4+ T cells to TH1 cells. Nevertheless, anti-ribosomal P Abs have been associated specifically with membranous LN, as opposed to the TH1-induced proliferative LN (Hirohata 2011). Another mechanism by which anti-ribosomal P Abs may contribute to the pathogenesis of SLE is through their ability to penetrate into cells, disturbing cellular physiologic functions such as protein synthesis and leading to apoptosis. Cellular penetration is thought to be mediated by a membrane form of the P0 ribosomal protein. This process was demonstrated in vitro in HepG2 cells, and was postulated to underlie the pathogenesis of a number of autoimmune hepatic diseases. Furthermore, treatment with monoclonal anti-ribosomal P Abs promoted apoptosis of Jurkat T cells in vitro. Thus, anti-ribosomal P Abs may be involved in the pathogenesis of the lymphocytic dysfunction in SLE (Toubi and Shoenfeld 2007). Currently, however, there is no direct evidence of the ability of anti-ribosomal P Abs to penetrate into renal cells, and it is yet unknown if cellular penetration is relevant to LN pathogenesis.

Conclusion

SLE is characterized by the expression of numerous autoantibodies, only a subset of which is pathogenic and contributes directly to target organ damage. Lupus nephritis, one of the major causes of morbidity and mortality in SLE, is assumed to be initiated and perpetuated by an autoimmune response mediated via these pathogenic autoAbs. A number of mechanisms have been proposed for autoAb-mediated LN, including in situ immune complex deposition, cross-reactivity of anti-dsDNA Abs with glomerular antigen, direct cell activation and/or cytotoxicity, and antibody internalization. Nevertheless, while binding to DNA in the specificity most closely associated with LN, and anti-dsDNA autoAbs most probably play an important role in the nephritic process, injection of purified Abs only rarely induces overt histological damage, or reproduces the complete histological picture associated with proliferative LN. Therefore, it is important to recognize that anti-dsDNA or other nephritogenic antibodies are often a necessary, but not always sufficient component in the various pathways contributing to full expression of lupus nephritis.

Cross-References

- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Pathogenesis

References

Aranow C, Zhou D, Diamond B. Anti-DNA antibodies: structure, regulation and pathogenicity. In: Lahita RG, Tokos G, Buyon J, Koike T, editors. Systemic lupus erythematosus. 5th ed. London: Elsevier; 2011. p. 235–58.

Chindalore V, Neas B, Recihlin M. The association between anti-ribosomal P antibodies and active nephritis in systemic lupus erythematosus. Clin Immunol Immunopathol. 1998;87:292–6.

Coremans IE, Spronk PE, Bootsma H, Daha MR, van der Voort EA, Kater L, et al. Changes in antibodies to C1q predict renal relapses in systemic lupus erythematosus. Am J Kidney Dis. 1995;26:595–601.

Deocharan B, Qing X, Lichauco J, Putterman C. Alpha-actinin is a cross-reactive renal target for pathogenic anti-DNA antibodies. J Immunol. 2002;15:3072–8.

Ghirardello A, Villalta D, Morozzi G, Afeltra A, Galeazzi M, Gerli R, et al. Evaluation of current methods for the

measurement of serum anti double-stranded DNA antibodies. Ann NY Acad Sci. 2007;1109:401–6.
Hanrotel-Saliou C, Segalen I, Le Meur Y, Youinou P, Renaudineau Y. Glomerular antibodies in lupus nephritis. Clin Rev Allergy Immunol. 2011;40:151–8.
Hirohata S. Anti-ribosomal P, antibodies and lupus nephritis. Clin Exp Nephrol. 2011;15:471–7.
Kalaaji M, Mortensen E, Jørgensen L, Olsen R, Rekvig OP. Nephritogenic lupus antibodies recognize glomerular basement membrane-associated chromatin fragments released from apoptotic intraglomerular cells. Am J Pathol. 2006;168:1779–92.
Kallenberg CGM. Anti-C1q autoantibodies. Autoimmun Rev. 2008;7:612–5.
Krishnan MR, Wang C, Marion T. Anti-DNA auto-antibodies initiate experimental lupus nephritis by binding directly to the glomerular basement membrane in mice. Kidney Int. 2012;82:184–92.
Mannik M, Wener M. Deposition of antibodies to the collagen-like region of C1q in renal glomeruli of patients with proliferative lupus glomerulonephritis. Arthritis Rheum. 1997;40:1504–11.
Mostoslavsky G, Fischel R, Yachimovich N, Yarkoni Y, Rosenmann E, Monestier M, et al. Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry. Eur J Immunol. 2001;31:1221–7.
Putterman C. New approaches to the renal pathogenicity of anti-DNA antibodies in systemic lupus erythematosus. Autoimmun Rev. 2004;3:7–11.
Reichlin M, Wolfson-Reichlin M. Correlations of anti-dsDNA and anti-ribosomal P autoantibodies with lupus nephritis. Clin Immunol. 2003;108:69–72.
Rekvig OP, Putterman C, Casu C, Gao HX, Ghirardello A, Mortensen ES, Tincani A, Doria A. Autoantibodies in lupus: culprits or passive bystanders? Autoimmun Rev. 2011; doi:10.1016/j.autrev.2011.10.021.
Renaudineau Y, Deocharan B, Jousse S, Renaudineau E, Putterman C, Youinou P. Anti-alpha-actinin antibodies: a new marker of lupus nephritis. Autoimmun Rev. 2007;6:464–8.
Rovin BH, Stillman IE. Kidney. In: Lahita RG, Tokos G, Buyon J, Koike T, editors. Systemic lupus erythematosus. 5th ed. London: Elsevier; 2011. p. 769–814.
Sinico RA, Rimoldi L, Radice A, Bianchi L, Gallelli B, Moroni G. Anti-C1q autoantibodies in lupus nephritis. Ann NY Acad Sci. 2009;1173:47–51.
Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1982;25:1271–7.
Toubi E, Shoenfeld Y. Clinical and biological aspects of anti-P ribosomal protein autoantibodies. Autoimmun Rev. 2007;6:119–25.
Trouw LA, Groeneveld TW, Seelen MA, Duijs JM, Bajema IM, Prins FA, et al. Anti-C1q autoantibodies deposit in glomeruli but are only pathogenic in combination with glomerular C1q-containing immune complexes. J Clin Invest. 2004;114:679–88.
Tsokos GC. Mechanisms of disease: systemic lupus erythematosus. N Engl J Med. 2011;365:2110–21.
Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol. 2004;15:241–50.
Youinou P, Putterman C. The role of anti-α-actinin antibodies in the pathogenesis and monitoring of lupus nephritis, editorial. Arthritis Res Ther. 2009;11:137.
Yung S, Cheung KF, Zhang Q, Chan TM. Anti-dsDNA antibodies bind to mesangial annexin II in lupus nephritis. J Am Soc Nephrol. 2010;21:1912–27.
Zhao Z, Weinstein E, Tuzova M, Davidson A, Mundel P, Marambio P, et al. Cross-reactivity of human lupus anti-DNA antibodies with alpha-actinin and nephritogenic potential. Arthritis Rheum. 2005;52:522–30.

Neutrophilic Dermatoses: Leukocytoclastic Vasculitis

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Synonyms

Cutaneous small-vessel vasculitis; Cutaneous vasculitis; Hypersensitivity angiitis; Hypersensitivity vasculitis; Leukocytoclastic vasculitis; Small-vessel vasculitis; Vasculitis

Definition

Leukocytoclastic vasculitis is inflammation of the small blood vessels of the skin. It may occur alone or in association with a number of disease states and clinically results in characteristic skin lesions which are non-blanching and often on the lower extremities.

Background

Vasculitis refers to inflammation of blood vessel walls. This inflammation often leads to vessel destruction and subsequent ischemic damage of the organs supplied. This inflammation can affect multiple organs, but those with dense vascular supply such as the gastrointestinal tract, kidneys, lungs, and skin are the most severely clinically affected. Leukocytoclasia is a histopathological term referring to the presence of breakdown of neutrophils. The term leukocytoclastic vasculitis (LCV) is commonly interchangeably used to refer to cutaneous small-vessel vasculitis, which is the subject of this review, as it is the primary histologic finding in this condition although to be strictly precise leukocytoclasia can occur to some degree in any vasculitis. Similarly although the synonyms provided above are frequently used to refer to the same condition, they are not strictly identically defined, and readers should be careful when accessing the literature.

There are a variety of classification standards for further subdividing the different types of vasculitis (Luqmani et al. 2011). Although these differ somewhat, most incorporate the size of vessel involved, whether small-, medium-, or large-vessel vasculitis, as primary categorization criteria. As already noted "leukocytoclastic vasculitis" is a vasculitis of small vessels. In the skin this constitutes the arterioles and postcapillary venules of the papillary and mid-dermis.

Clinical Presentation

Skin lesions are most common on the lower legs but any area can be affected (Goldsmith and Fitzpatrick 2012). Lesions are usually small (1–3 mm) and round, although they may coalesce to form plaques. They are subtly palpable and are non-blanching, thus being described as purpura. In the early stages, lesions may be asymptomatic, itchy, or burning. Inflammation is particularly intense or persistent plaques can form areas of necrosis and ulceration and this can be intensely painful. This, as well as more retiform purpura, is more common in Henoch-Schönlein purpura (Goldsmith and Fitzpatrick 2012; Piette and Stone 1989).

Other organs may also be affected in LCV with the most common being the joints, kidney, and gastrointestinal tract. LCV may be acute, chronic, or recurrent. Chronic and recurrent forms are more commonly associated with an underlying systemic disease. Known triggers of LCV include infections, inflammatory disorders, malignancies, autoimmune diseases, and medications (see below). Despite these many associations, between 30 % and 50 % of cases remain idiopathic. Prognosis is best in those with acute LCV limited only to the skin and unassociated with any systemic disease.

Histology

Histology depends on the timing of the biopsy (McKee et al. 2005). In early or late lesions, characteristic changes may not be present with early lesions showing little change, whereas late lesions show a more mixed infiltrate and, if ulceration has occurred, extensive necrosis. Characteristic histology shows dense perivascular infiltration of neutrophils with invasion of the vessel walls by neutrophils. There is leukocytoclasia (neutrophil fragments), endothelial swelling, fibrinoid necrosis of the vessel walls, and often extravasation of erythrocytes (red blood cells). Eosinophils suggest urticarial vasculitis or drug-associated disease.

In Henoch-Schönlein purpura, immunofluorescent staining shows IgA deposits which can be helpful diagnostically. In other types of cutaneous vasculitis, immunofluorescent stains are not often clinically helpful but frequently show immunoglobulins (immunoglobulin G, immunoglobulin M) and complement components (e.g., C3, C4) deposited on the skin basement membrane. In one study, direct immunofluorescence was positive for at least one reactant in 62 % of cases with the most common deposit being C3 followed by IgG, IgA, and IgM (Khetan et al. 2012).

Immunology and Pathogenesis

There is good evidence, including the immunostaining findings above, to support the idea that most cutaneous vasculitis are pathogenically associated with deposition of circulating immune complexes. Individual triggers for this process vary and the initiation of this process is likely different for each. However, it seems clear that after the initial insult, a common pathway is followed in the majority of LCV where deposition of these circulating immune complexes into the vessel wall results in activation of the complement cascade, release of histamines and other vasoactive proteins leading to vessel leakage and erythrocyte extravasation, and cytokine release which recruits neutrophils to the site resulting in vessel damage.

In contrast, in conditions such as Wegener's granulomatosis, there is a strong association with ANCA recognizing epitopes on the neutrophil enzymes proteinase 3 and myeloperoxidase. These enzymes are confined neutrophil granules and reach the plasma membrane after specific stimuli, although for proteinase 3, a genetically encoded pattern may regulate expression in the absence of detectable stimuli (Kallenberg 2008). These ANCA promote deregulated inflammatory clearance of neutrophils, possibly directly resulting in leukocytoclasia. While LCV is not highly associated with ANCAs, LCV can occur in the context of systemic vasculitis as in Wegner's, and this may therefore be one mechanism for its formation.

Another factor which may be important in LCV is the host's ability to produce the prototypic long pentraxin 3 (PTX3). PTX3 is produced by inflammatory cells, including neutrophils, and tissues in response to inflammatory signals and TLR activation (Manfredi et al. 2008). Elevated PTX3 has been identified in both mouse models and human studies in patients with vascular damage from atherosclerotic disease or preeclampsia. PTX3 is believed to be protective and reduces the inflammation and effect of cytotoxic signals. This is reflected in experiments with PTX3-deficient mice which have an exacerbated damage after reperfusion. PTX3 has also been shown to be produced in small-vessel vasculitis lesions by endothelial and mononuclear cells (van Rossum et al. 2006). Patients with vasculitis had significantly higher concentrations of PTX3, and these levels could be lowered with immunosuppressive treatments (Fazzini et al. 2001). Finally, there is some evidence that the cross talk between activated platelets and circulating leucocytes may be disturbed in LCV (Maugeri et al. 2009; Tervaert 2009).

Epidemiology, Etiology, and Associated Conditions

LCV shows no apparent bias by gender, age, or ethnicity (Grzeszkiewicz and Fiorentino 2006).

The list of conditions and medications associated with LCV is broad, and these are therefore best broken down into categories. In bacterial infections, upper respiratory tract infections, particularly with beta-hemolytic streptococci, and gastrointestinal infections are implicated most often, but bacterial endocarditis can elicit an LCV. Of viral infections hepatitis B and C are commonly associated, with hepatitis C likely pathogenically linked through the presence of cryoglobulins. More common infections such as EBV have also been associated. Both HIV and its treatments are associated with LCV.

The most common drugs that can cause cutaneous vasculitis are antibiotics, particularly beta-lactam drugs, and whether an antibiotic or the original infection is the trigger is sometimes difficult to establish. Other common medications linked to LCV include nonsteroidal anti-inflammatory drugs and diuretics; however, the list of drugs that are potential causes is extensive (Doyle and Cuellar 2003). In addition to medications, other exogenous agents such as toxins, industrial chemicals, and even foods and food additives may cause vasculitis. Collagen-vascular diseases account for 10–15 % of vasculitis cases with rheumatoid arthritis and Sjögren syndrome having the highest association.

Inflammatory bowel disease may also be associated with cutaneous vasculitis. Malignancy accounts for less than 1 % of cases of cutaneous vasculitis.

The most common association of these is with lymphoproliferative diseases. In these cases treatment of the underlying malignancy is often curative, and LCV activity can track onset and recurrence. However, in some patients the immune activation is persistent independent of the underlying cause.

Cutaneous vasculitis may also be part of a larger-vessel vasculitis most commonly those that have mixed involvement of small and medium vessels such as Wegener's and microscopic polyarteritis but polyarteritis nodosa and Churg-Strauss syndrome can also demonstrate LCV at times in their course.

Work-Up

Given the broad differential work-up is often extensive but should begin with a full review of systems and detailed disease, medical, and medication history. Serologies should include complete blood cell count, erythrocyte sedimentation rate, and blood chemistry panel, and due to the risk of kidney involvement, a urinalysis should be performed. Chest x-ray and stool guaiac are often obtained as screenings. Antistreptolysin antibodies or cultures for URI or GI infections may be performed if clinically suggested. If no obvious cause (preceding upper respiratory infection) is present, further work-up should be guided by other clinical signs and personal and family medical history.

In patients suspected of a connective tissue disease, ANA, dsDNA, Ro/La, RNP, RF, and complement levels should be obtained, including total hemolytic complement (CH100 or CH50), C3 levels, and C4 levels. The latter should also be considered in cases of urticarial vasculitis. Serum protein electrophoresis and immunofixation should be checked in any patient where connective tissue or malignancy is of concern, as well as cryoglobulins, which should also be drawn if hepatitis B and C antibody is considered. Where further organ involvement is suspected, antinuclear antibody cANCA and pANCA should also be obtained. In sicker, febrile patients, cardiac ultrasonography and blood cultures should be considered. HIV testing should be performed in patients with risk factors.

A rigorous search for underlying malignancy is not usually pursued unless there is some clinical indication (e.g., weight loss, strong family history, toxic exposure risk), but what specific testing is obtained is often guided by that indication.

It is also recommended that pulmonary function tests should be obtained in patients with hypocomplementemic urticarial vasculitis.

Treatment and Prognosis

Initial treatment is guided by identification of the underlying cause, e.g., treatment of infections or withdrawal of medication. Symptomatic treatment involves elevation of dependent areas and simple wound care when skin breakdown occurs. Itch may be treated with sedating antihistamines.

Corticosteroids either topically, intralesionally, or systemically can provide rapid improvement in some varieties of LCV. In addition, colchicine and dapsone are considered first-line treatments for persistent cases of LCV and other neutrophilic dermatosis. Other agents that have been used with varying success include cyclosporine, potassium iodide, indomethacin, clofazimine, thalidomide, and CellCept, as well as biologic agents such as infliximab and etanercept, although LCV has also been reported as occurring secondary to TNF-alpha antagonist agents. Rituximab has also shown good promise for treatment in otherwise recalcitrant disease (Chung et al. 2006; Chung and Seo 2009).

While mostly benign and limited in duration, LCV can be chronic and can have severe complications both cutaneous and systemic. In these latter cases, it requires ongoing treatment for suppression.

Cross-References

- ▶ Neutrophilic Dermatoses: Pyoderma Gangrenosum
- ▶ Neutrophilic Dermatoses: Sweet's Syndrome

References

Chung SA, Seo P. Advances in the use of biologic agents for the treatment of systemic vasculitis. Curr Opin Rheumatol. 2009;21(1):3–9.

Chung L, et al. Successful use of rituximab for cutaneous vasculitis. Arch Dermatol. 2006;142(11):1407–10.

Doyle MK, Cuellar ML. Drug-induced vasculitis. Expert Opin Drug Saf. 2003;2(4):401–9.

Fazzini F, et al. PTX3 In small-vessel vasculitides: an independent indicator of disease activity produced at sites of inflammation. Arthritis Rheum. 2001;44(12):2841–50.

Goldsmith LA, Fitzpatrick TB. Fitzpatrick's dermatology in general medicine. New York: McGraw-Hill Professional; 2012.

Grzeszkiewicz TM, Fiorentino DF. Update on cutaneous vasculitis. Semin Cutan Med Surg. 2006;25(4):221–5.

Kallenberg CG. Pathogenesis of PR3-ANCA associated vasculitis. J Autoimmun. 2008;30(1–2):29–36.

Khetan P, et al. An aetiological & clinicopathological study on cutaneous vasculitis. Indian J Med Res. 2012;135:107–13.

Luqmani RA, et al. Nomenclature and classification of vasculitis - update on the ACR/EULAR diagnosis and classification of vasculitis study (DCVAS). Clin Exp Immunol. 2011;164(Suppl 1):11–3.

Manfredi AA, et al. Pentraxins, humoral innate immunity and tissue injury. Curr Opin Immunol. 2008;20(5):538–44.

Maugeri N, et al. Translational mini-review series on immunology of vascular disease: mechanisms of vascular inflammation and remodelling in systemic vasculitis. Clin Exp Immunol. 2009;156(3):395–404.

McKee PH, Eduardo C, Scott RG. Pathology of the skin: with clinical correlations. (Edited by McKee PH, Eduardo C, Scott RG). Edinburgh/Philadelphia: Elsevier Mosby; 2005.

Piette WW, Stone MS. A cutaneous sign of IgA-associated small dermal vessel leukocytoclastic vasculitis in adults (henoch-schonlein purpura). Arch Dermatol. 1989;125(1):53–6.

Tervaert JW. Translational mini-review series on immunology of vascular disease: accelerated atherosclerosis in vasculitis. Clin Exp Immunol. 2009;156(3):377–85.

van Rossum AP, et al. Abundance of the long pentraxin PTX3 at sites of leukocytoclastic lesions in patients with small-vessel vasculitis. Arthritis Rheum. 2006;54(3):986–91.

Neutrophilic Dermatoses: Pyoderma Gangrenosum

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Synonyms

Brocq's geometric phagedenism; Geometric phagedenism; Neutrophilic dermatosis; PG; "Pyoderma ecthyma gangrenosum"; Pyoderma gangrenosum; Vegetans gangrenosum

Definition

Pyoderma gangrenosum is a skin condition characterized by deep painful ulcers with undermined borders. It is associated with many diseases but can occur independently and represents a dense inflammation of neutrophils.

Historical Background

Retrospectively identified as the same entity, Louis Brocq reported in 1908 a series of cases of pyoderma gangrenosum, which he called "geometric phagedenism" to reflect the circular geometric appearance and rapid consumption of tissue (phageton [Greek], meaning food, consumption) (Farhi et al. 2008). Although such reports may be retrospectively identified as pyoderma gangrenosum (PG), the original report that used this name and identified this as an entity was from the Mayo Clinic Department of Dermatology in 1930. In that report Drs. Brunsting, Goeckerman, and O'Leary identified five patients with classic PG, four of

which had ulcerative colitis and one of which had chronic idiopathic purulent pleurisy. The word pyoderma was adopted as they believed this was due to a distant infectious process. It was not until later that the idea that this is an inflammatory reaction and not infectious was accepted, but by that time the term pyoderma gangrenosum had become established.

Classification and Clinical Presentation

PG is often classified into one of the four clinical variants depending on its appearance: classical (or ulcerative) PG, pustular PG, bullous PG, and vegetative PG. Classic or ulcerative PG skin lesions often start as a small papule or sterile pustule that then breaks down to form an ulcer. The ulcer is often deep, with a characteristic well-circumscribed, violaceous, undermined border that can expand rapidly. The lesions are often intensely painful and even after healing often leave cribriform scars. Lesions favor the lower extremities with the pretibial area most common, and peristomal (15 %) the second, but have been reported in almost all body locations. Lesions on the trunk can be induced by surgery with pathergy (new lesions at the site of minor trauma) occurring in 25–50 % of cases (Brooklyn et al. 2006).

In pustular PG the lesions do not form ulcers but instead persist at the pustular stage for months at a time. These lesions are also intensely painful but lead to less scarring and tend to occur with greater frequency on the trunk and extensor surfaces. It is more common in inflammatory bowel disease (IBD) patients.

Bullous PG in contrast more commonly occurs on the face and upper extremities. The bullae spread concentrically and, although may break down into ulcers with undermined edges, tend to be more superficial. This form of PG is more common in patients with a hematological malignancy.

Vegetative PG is usually a less aggressive form of the disease. It tends not to spread as rapidly and usually remains limited to one or a few locations. It also responds more readily to treatment.

In all forms patients may also exhibit systemic symptoms and appear sick with fever (>38 °C) as the most common non-cutaneous symptom, but malaise, headache, arthralgia, and myalgia may also occur. This may be concurrent or precede skin findings by days to weeks.

In addition to the skin, PG can affect many other systems producing diverse symptoms. Reports have been made of ocular lesions (Happle et al. 1977), sterile pulmonary nodules (Vignon-Pennamen et al. 1989), involvement of the liver and spleen (Vadillo et al. 1999), and myositis (Marie et al. 2001), as well as by direct extension to the bone.

Histology

The histology of PG depends in part on the timing of the biopsy. In very early PG inflammation may be of a mixed-cell infiltrate that is primarily in the upper dermis. However, this is soon replaced by intense neutrophilic infiltration, hemorrhage, and necrosis of the overlying epidermis. Although neutrophils are often around and occasionally within the vessel walls due to the density of the infiltrate, leukocytoclastic vasculitis, which would be indicated by fibrin deposition and neutrophils within vessel walls, is only present in 40 % of cases (Lever and Elder 2009). Histology may be supportive of a diagnosis of PG but is not diagnostic.

Diagnostic Criteria

With no set diagnostic criteria and nonspecific histology, diagnosis of PG is by exclusion. The differential diagnosis includes other neutrophilic dermatoses; vascular occlusive diseases; calciphylaxis; vasculitis; Wegener's granulomatosis; antiphospholipid syndrome; malignancy, most commonly squamous but also locally invasive or metastatic malignancies; infections, most notably deep fungal such as sporotrichosis or atypical mycobacterial infections, in the postsurgical patient gangrene or suppurative bacterial infections, cutaneous tuberculosis,

late syphilis, or deep herpetic infections; traumatic ulceration; factitial disease; arthropod assault, most notably arachnid; and non-PG forms of drug reactions. This differential is broad and can often be significantly narrowed based on a detailed history, with information on the patient's medical conditions, timing, and location of the lesion.

Epidemiology, Etiology, and Associated Conditions

Onset of classic PG is generally between the 2nd and 6th decade, with the incidence in the general population estimated at between 3 and 10 per million per year. It disproportionately affects women but shows no bias in ethnic background or geographic distribution (Wollina 2007).

As noted above PG can be associated with a variety of systemic illnesses. Classical PG is associated with inflammatory bowel disease; however, it may also be associated with underlying malignancy and can be drug induced. 10–15 % of PG is associated with ulcerative colitis and another 10–15 % with Crohn's (Wollina 2007). Despite this only 1–2 % of patients with IBD will suffer from PG (Bernstein et al. 2001). Despite the association with IBD, IBD-associated PG activity is unrelated to the activity of the underlying IBD disease. PG can also occur in association with hematological dyscrasias and malignancies, including leukemia, lymphoma, monoclonal gammopathy, and myelodysplastic syndrome. In contrast to IBD-associated PG, PG associated with malignancy often, but not always, marks the onset or recurrence of the underlying malignancy and is often put in remission by treatment of the underlying malignancy.

PG can also be associated with arthritis, both via association with standard rheumatological conditions such as psoriatic or rheumatoid arthritis and spondylitis, and as part of the PAPA syndrome (pyogenic arthritis, pyoderma gangrenosum, and acne) an autosomal dominant condition that can be caused by one of the two mutations in the PSTPIP1 gene on chromosome 15 that codes for CD2 binding protein 1 (Wise et al. 2002). In this latter condition, the arthritis usually presents first at a young age with the onset of skin findings at puberty. PG can rarely be associated with autoimmune diseases such as lupus erythematosus or Sjögren's syndrome.

Although PG has been reported in association with infections, it is usually in association with infections causing chronic inflammation. The classic example of this is in chronic hepatitis C infection (Smith et al. 1996) although it can also occur during hepatitis C treatment (Yurci et al. 2007), and reports of PG in association with colonic (Kim et al. 1994) and testicular forms (Anton Botella et al. 1989) of tuberculosis exist.

A variety of drugs have also been associated with inducing PG including isotretinoin (Gangaram et al. 1997), propylthiouracil (Darben et al. 1999), granulocyte colony-stimulating factor (Ross et al. 1991), and oncologic medications such as sunitinib (ten Freyhaus et al. 2008).

In addition to the above associations, there are many untested or single case report associations in the literature.

Work-Up

Given the above disease associations, a rigorous work-up is generally recommended but should be guided by the differential generated by the detailed history. Work-up therefore begins with a detailed history and physical. Lesional skin biopsy is sometimes warranted but is more useful for diagnosing items from the differential than PG itself. A complete blood cell count with leukocyte differential and platelet count should be performed, serum chemistries including liver function tests for evaluation of hepatic function and creatinine and a urinalysis for evaluation of renal function, and consideration of acute phase reactants such as the erythrocyte sedimentation rate or C-reactive protein for tracking of disease activity. Serologic hepatitis screening should be performed, and in the right clinical context, urine and fecal cultures, rheumatoid factor, and thyroid-stimulating hormone testing should also be performed.

Given the association with hematologic dyscrasias, serum and urine protein electrophoresis, peripheral smear, and bone marrow aspiration or biopsy should be considered where appropriate. In addition to the above, where cancer is suspected, it has also been suggested that fecal occult blood smear, carcinoembryonic antigen level, pap test in women, chest x-ray, as well as age- and symptom-appropriate cancer screenings such as endometrial biopsy in postmenopausal women with spotting, breast exam or mammogram, testicular and prostate exam, and colonoscopy in patients over 50 years of age be performed. Colonoscopy is also warranted when IBD symptoms are present, but a diagnosis has not as yet been made.

If infectious processes are in the differential, tissue cultures of the ulcer for bacteria, fungi, atypical mycobacteria, and viruses are needed.

Where traumatic or veno-occlusive disease is considered, x-ray or Doppler studies may be warranted.

Treatment and Prognosis

If an underlying cause can be identified, then treatment of the underlying cause, including chemotherapy of underlying malignancy, treatment of Hepatitis C, or withdrawal of the inciting medication, can be curative of the PG. Other than resection of tumor, surgical intervention plays little role in the treatment of PG given its propensity for pathergy and the inherent potential therefore of worsening the condition.

Corticosteroids either topically, intralesionally, or systemically can often provide rapid improvement in all varieties of PG (Wollina 2007). In addition, infliximab, cyclosporine, potassium iodide, colchicine, and dapsone may also be considered first-line therapies for this and other neutrophilic dermatosis. Other agents that have been used with varying success include indomethacin, clofazimine, thalidomide, and CellCept, as well as biologic agents such as etanercept (Yamauchi et al. 2006) although PG has also been reported as occurring secondary to TNF-alpha antagonist agents.

While mostly benign, PG can have severe complications and require ongoing treatment for suppression. Even after remission, PG may recur regardless of whether the remission was spontaneous or therapy induced.

Cross-References

- ▶ Neutrophilic Dermatoses: Leukocytoclastic Vasculitis
- ▶ Neutrophilic Dermatoses: Sweet's Syndrome

References

Anton Botella F, et al. Pyoderma gangrenosum associated with testicular tuberculosis. An Med Interna. 1989;6(10):549.

Bernstein CN, et al. The prevalence of extraintestinal diseases in inflammatory bowel disease: a population-based study. Am J Gastroenterol. 2001; 96(4):1116–22.

Brooklyn T, Dunnill G, Probert C. Diagnosis and treatment of pyoderma gangrenosum. BMJ. 2006;333(7560):181–4.

Darben T, et al. Pyoderma gangrenosum with secondary pyarthrosis following propylthiouracil. Australas J Dermatol. 1999;40(3):144–6.

Farhi D, Wallach D, Avril MF. Pyoderma gangrenosum is 100 years old. From Louis brocq to biotherapies. Rev Prat. 2008;58(4):457–61.

Gangaram HB, et al. Pyoderma gangrenosum following treatment with isotretinoin. Br J Dermatol. 1997;136(4):636–7.

Happle R, Schiffer HP, Kovary PM. Ocular involvement in pyoderma gangrenosum. Arch Dermatol. 1977;113(11):1612.

Kim NY, et al. Pyoderma gangrenosum in a patient with colonic tuberculosis. Am J Gastroenterol. 1994;89(8): 1257–9.

Lever WF, Elder DE. Lever's histopathology of the skin. Philadelphia: Lippincott Williams & Wilkins; 2009.

Marie I, et al. Neutrophilic myositis as an extracutaneous manifestation of neutrophilic dermatosis. J Am Acad Dermatol. 2001;44(1):137–9.

Ross HJ, et al. Bullous pyoderma gangrenosum after granulocyte colony-stimulating factor treatment. Cancer. 1991;68(2):441–3.

Smith JB, et al. Pyoderma gangrenosum in a patient with cryoglobulinemia and hepatitis C successfully treated with interferon Alfa. J Am Acad Dermatol. 1996;34(5 Pt 2):901–3.

ten Freyhaus K, et al. Pyoderma gangrenosum: another cutaneous side-effect of sunitinib? Br J Dermatol. 2008;159(1):242–3.

Vadillo M, et al. Pyoderma gangrenosum with liver, spleen and bone involvement in a patient with chronic myelomonocytic leukaemia. Br J Dermatol. 1999;141(3):541–3.
Vignon-Pennamen MD, et al. Pyoderma gangrenosum with pulmonary involvement. Arch Dermatol. 1989;125(9):1239–42.
Wise CA, et al. Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. Hum Mol Genet. 2002;11(8):961–9.
Wollina U. Pyoderma gangrenosum – a review. Orphanet J Rare Dis. 2007;2:19.
Yamauchi PS, et al. Treatment of recurrent Sweet's syndrome with coexisting rheumatoid arthritis with the tumor necrosis factor antagonist etanercept. J Am Acad Dermatol. 2006;54(3 Suppl 2): S122–6.
Yurci A, et al. Pyoderma gangrenosum and exacerbation of psoriasis resulting from pegylated interferon alpha and ribavirin treatment of chronic hepatitis C. Eur J Gastroenterol Hepatol. 2007;19(9):811–5.

Neutrophilic Dermatoses: Sweet's Syndrome

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Synonyms

Acute febrile neutrophilic dermatosis; Gomm-Button disease; Sweet's syndrome

Definition

Sweet's is a condition in which dense neutrophil inflammation leads to abrupt onset of tender edematous papules and plaques on the skin, often accompanied by systemic symptoms such as fever and malaise and an elevation in white cell count.

Historical Background

Although prior reports (Costello et al. 1955) have been retrospectively identified as Sweet's syndrome (SS), the original report that identified this as an entity was in 1964, when Dr. Robert Douglas Sweet reported a series of eight women with the disease (Sweet 1964). Originally called Gomm-Button disease after the first two patients who were diagnosed with it, Dr. Sweet instead recommended that the disease be named descriptively and thus suggested the diagnostic moniker "acute febrile neutrophilic dermatosis." Despite this, the disease is popularly known as Sweet's syndrome after Dr. Sweet the author of the first report.

Classification and Clinical Presentation

Sweet's is often divided into classical, malignancy-associated and drug-induced forms of the disease, dependent on the underlying etiology. In all forms patients may appear very sick with fever (>38 °C), the most common non-cutaneous symptom. This may precede skin findings by days to weeks. Typical symptoms associated with high fevers such as malaise and headache are also common but arthralgia and myalgia may also occur. The skin lesions may be single or multiple and favor the upper extremities, face, and neck. They are red-brown to purple in color and often form raised edematous papules and plaques. Lesions may appear pseudo-vesiculate or ulcerate, although this is more common in the malignancy-associated variant, and in persistent lesions central clearing can lead to arcuate formations. Pathergy is characteristic with new lesions occurring at the sites of minor trauma. Oral mucosal lesions are rare but more common in Sweet's associated with hematologic malignancies. In contrast, ocular lesions are more common in classical Sweet's.

Clinical variants of Sweet's that have been reported include a pustular form, subcutaneous Sweet's characterized by erythema nodosum-like erythematous, tender dermal nodules on the extremities, and neutrophilic dermatosis of the dorsal hands, although some authors separate this

final entity from Sweet's by including in its definition the presence of a leukocytoclastic vasculitis.

In addition to the skin, Sweet's can affect many other systems producing diverse symptoms (Cohen and Kurzrock 2003), including psychiatric and neurologic (neuro-Sweet disease, encephalitis, aseptic meningitis, polyneuropathy), ophthalmologic and auricular (conjunctivitis, dacryoadenitis, episcleritis, glaucoma, iridocyclitis, iritis, retinitis, uveitis, idiopathic progressive bilateral sensorineural hearing loss), cardiovascular (aortic stenosis, aortitis, cardiomegaly, coronary artery occlusion, heart failure), pulmonary (pleural effusion and upper airway obstruction), renal (mesangiocapillary glomerulonephritis), gastrointestinal (ileal infiltrate, pancolitis), hepatosplenomegaly, and musculoskeletal systems (sterile arthritis and osteomyelitis, myositis, fasciitis, myalgias, tendinitis, and tenosynovitis).

Histology

Sweet's syndrome is characterized by a dense, diffuse infiltrate of mature neutrophils typically located in the papillary and upper reticular dermis with marked edema. In hematologic malignancy-related Sweet's skin lesions, leukemia cutis may be present, and in drug-induced Sweet's, occasional eosinophils may be noted. However, rare lymphocytes, histiocytes, and eosinophils may be noted in all varieties. Karyorrhexis or leukocytoclasia (fragmented neutrophil nuclei), swollen endothelial cells, and dilated small blood vessels may also be present. However, the overlying epidermis is normal, unless ulceration has occurred, and leukocytoclastic vasculitis, which would be indicated by fibrin deposition and neutrophils within vessel walls, is absent (Lever and Elder 2009).

Diagnostic Criteria

Diagnostic criteria, which depend on the subcategory of Sweet's, were first established in 1986 (Su and Liu 1986) with modification adopted in 1994 (von den Driesch 1994) and for drug-induced Sweet's in 1996 (Walker and Cohen 1996). The criteria include (1) abrupt onset of classical cutaneous lesions; (2) dense neutrophilic infiltrate on histology but without any evidence of leukocytoclastic vasculitis; (3) pyrexia $>38\,^{\circ}C$; (4) in malignancy-associated Sweet's association with an underlying hematologic or visceral malignancy, in classical Sweet's association with an inflammatory disease or pregnancy or preceded by an upper respiratory or gastrointestinal infection or vaccination, or in drug-induced Sweet's temporal relation to the administration of drug and its rechallenge; (5) excellent response to treatment with corticosteroids; (6) and three of the following: erythrocyte sedimentation rates >20 mm/h, positive C-reactive protein, $>8{,}000$ leukocytes, and >70 % neutrophils at presentation.

Epidemiology, Etiology, and Associated Conditions

Onset of classic Sweet's is generally between the fourth and seventhdecade, although patients as young as 10 days old have been reported (Parsapour et al. 2003). It disproportionately affects women but shows no bias in ethnic background or geographic distribution. Classic Sweet's is most commonly associated with upper respiratory tract (streptococcosis) and gastrointestinal tract infections (salmonellosis and yersiniosis), inflammatory bowel disease (both ulcerative colitis and Crohn's disease), and pregnancy.

A review of published studies suggested that 21 % of Sweet's cases have an underlying associated malignancy (Cohen and Kurzrock 1993). Malignancy-associated Sweet's occurs equally in men and women. The most common associated malignancy is acute myelogenous leukemia, and the most common solid-tumor malignancies are carcinomas of the genitourinary organs, breast, and gastrointestinal tract (Cohen 2007). Sweet's can predate, postdate, or be simultaneous with the discovery of the cancer or its relapse. Hematologic malignancies have been reported as long as 11 years after the first onset of Sweet's.

Many drugs from many different drug classes have been associated with drug-induced Sweet's. The most commonly associated is granulocyte colony-stimulating factor. The full range of causative drugs is reviewed elsewhere (Cohen 2007).

In addition to the above associations, there are many untested or single case report associations in the literature. Of these the most commonly reported associations include erythema nodosum, relapsing polychondritis, rheumatoid arthritis, sarcoidosis, and thyroid disease, including both Grave's disease and Hashimoto's thyroiditis (Cohen 2007).

Work-Up

Given the above diagnostic criteria and disease associations, a rigorous work-up is generally recommended. This should include a detailed history and physical exam, lesional skin biopsy, a complete blood cell count with leukocyte differential and platelet count, serum chemistries including liver function tests for evaluation of hepatic function and creatinine and a urinalysis for evaluation of renal function, and consideration of acute-phase reactants such as the erythrocyte sedimentation rate or C-reactive protein for tracking of disease activity. In the right clinical context, antistreptolysin-O antibody, urine and fecal cultures, rheumatoid factor, and thyroid stimulating hormone testing should also be performed.

In addition to the above, where cancer is suspected, it has also been suggested that fecal occult blood smear, carcinoembryonic antigen level, pap test in women, chest x-ray, and age- and symptom-appropriate cancer screenings such as endometrial biopsy in postmenopausal women with spotting, breast exam or mammogram, testicular and prostate exam, and colonoscopy in patients over 50 years of age be performed.

Treatment and Prognosis

If an underlying cause can be identified, then treatment of the underlying cause, including antibiotic treatment of gastrointestinal infections, delivery of pregnancy, surgical resection of a solid tumor, or withdrawal of the inciting medication, can be curative of the Sweet's. Other than resection of tumor, surgical intervention plays no role in the treatment of Sweet's given its propensity for pathergy and the inherent potential therefore of worsening the condition.

Corticosteroids either topically, intralesionally, or systemically by definition provide rapid improvement in all three varieties of Sweet's. In addition, potassium iodide (Horio et al. 1980) and colchicine (Suehisa and Tagami 1981) may also be considered first-line therapies for this dermatosis. Other agents that have been used with varying success include indomethacin, clofazimine, cyclosporin, and dapsone. Biologic agents, such as etanercept (Yamauchi et al. 2006), have also been used although Sweet's has also been reported as occurring secondary to TNF-alpha antagonist agents (Hawryluk et al. 2012).

While mostly benign, Sweet's can have severe complications both cutaneous and systemic and require ongoing treatment for suppression. Even after remission Sweet's syndrome may recur regardless of whether the remission was spontaneous or therapy induced, although recurrence is more common in malignancy-related Sweet's.

Cross-References

- ▶ Neutrophilic Dermatoses: Leukocytoclastic Vasculitis
- ▶ Neutrophilic Dermatoses: Pyoderma Gangrenosum

References

Cohen PR. Sweet's syndrome–a comprehensive review of an acute febrile neutrophilic dermatosis. Orphanet J Rare Dis. 2007;2:34.

Cohen PR, Kurzrock R. Sweet's syndrome and cancer. Clin Dermatol. 1993;11(1):149–57.

Cohen PR, Kurzrock R. Sweet's syndrome revisited: a review of disease concepts. Int J Dermatol. 2003;42(10):761–78.

Costello MJ, et al. Cutaneous manifestations of myelogenous leukemia. AMA Arch Derm. 1955;71(5):605–14.

Hawryluk EB, et al. Broad range of adverse cutaneous eruptions in patients on TNF-alpha antagonists. J Cutan Pathol. 2012;39(5):481–92.
Horio T, et al. Treatment of acute febrile neutrophilic dermatosis (Sweet's syndrome) with potassium iodide. Dermatologica. 1980;160(5):341–7.
Lever WF, Elder DE. Lever's histopathology of the skin. Philadelphia: Lippincott Williams & Wilkins; 2009.
Parsapour K, et al. Familial Sweet's syndrome in 2 brothers, both seen in the first 2 weeks of life. J Am Acad Dermatol. 2003;49(1):132–8.
Su WP, Liu HN. Diagnostic criteria for Sweet's syndrome. Cutis. 1986;37(3):167–74.
Suehisa S, Tagami H. Treatment of acute febrile neutrophilic dermatosis (Sweet's syndrome) with colchicine. Br J Dermatol. 1981;105(4):483.
Sweet RD. An acute febrile neutrophilic dermatosis. Br J Dermatol. 1964;76:349–56.
von den Driesch P. Sweet's syndrome (acute febrile neutrophilic dermatosis). J Am Acad Dermatol. 1994;31(4):535–56. quiz 557–60.
Walker DC, Cohen PR. Trimethoprim-sulfamethoxazole-associated acute febrile neutrophilic dermatosis: case report and review of drug-induced Sweet's syndrome. J Am Acad Dermatol. 1996;34(5 Pt 2):918–23.
Yamauchi PS, et al. Treatment of recurrent Sweet's syndrome with coexisting rheumatoid arthritis with the tumor necrosis factor antagonist etanercept. J Am Acad Dermatol. 2006;54(3 Suppl 2):S122–6.

Neutrophils

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Synonyms

Granulocytes; Polymorphonuclear leukocytes (PMN)

Definition

Neutrophils, which are produced in the bone marrow and circulate in the blood, are the most abundant type of white blood cells (leukocytes) in mammals and form an essential part of the innate immune system. Neutrophils comprise more than 50 % of the white blood cell population and during bacterial infection may increase to more than 80 %. The name neutrophil derives from staining characteristics on hematoxylin- and eosin-stained histological or cytological preparations. Whereas the intracytoplasmic granules of neutrophils stain a neutral pink, the other polymorphonuclear (multilobed nucleus) cells stain dark blue (basophils) or bright red (eosinophils). Polymorphonuclear leukocytes (PMN) are circulating surveillance cells, scanning for infection or other inflammatory events, to which they respond within minutes (Borregaard 2010).

Activation and Function

To get to the place where they are needed, the neutrophils must sense the presence of "danger" from a distance. They first adhere to and pass through the endothelium of the postcapillary venules (diapedesis) and migrate along a chemical gradient of molecules (chemoattractants) to the source of the alarm (chemotaxis). Then, the neutrophils encounter the target, ingest it, and destroy it (activation). However, since too much neutrophil activation can result in tissue injury, activation must be regulated (Bokoch 1995). Cell surface receptors allow neutrophils to detect the chemoattractants, which include lipid mediators (leukotriene B4, platelet-activating factor), proteins, and peptides (interferon gamma, interleukin-8, the complement split product C5a, formylated peptides). Other lipids such as the eicosanoids prostaglandin J2, lipoxin A4, and resolvins act as stop signals and prevent neutrophil-induced tissue injury by suppressing neutrophil migration and increasing neutrophil death (apoptosis) and facilitate resolution of inflammation (Serhan et al. 2008). Inflammation is a primitive protective response, but it is impairment of its resolution that can lead to tissue damage.

Phagocytosis and Degranulation

Ingestion by the neutrophil of the target particle or bacterium (phagocytosis) requires direct contact

with the opsonized (from the Greek "to prepare for the table") target, that is, coated with immunoglobulin and/or complement components (Caron and Hall 1998). Phagocytosis involves extension of the neutrophil membrane and invagination of the neutrophil at the point of contact to surround the target to form a phagocytic vacuole. Two forms of degranulation can then take place. The enzyme-laden granules (lysosomes) can fuse with the neutrophil plasma membrane and spill their contents into the extracellular space (exocytosis) or fuse with the phagocytic vacuole to form a phagolysosome. Exocytosis favors mobilization of lighter granules (secretory and specific granules), whereas in phagolysosome formation, fusion of azurophilic granules with the phagocytic vacuole results in the delivery of proteolytic enzymes, myeloperoxidase, and antibacterial proteins to the site of the ingested bacterium (Scher et al. 2013).

In addition to the proteases and other antibacterial proteins in their granules, neutrophils can kill bacteria through generation of toxic oxygen metabolites, a process termed the respiratory burst. The respiratory burst involves activation of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase localized to the membrane of specific granules. When assembled and activated, the NADPH transfers electrons from NADPH to generate superoxide, a reactive oxygen species. A subsequent dismutase reaction produces hydrogen peroxide which is converted to hypochlorous acid, an oxidant with potent bactericidal activity (DeLeo and Quinn 1996).

Production of Mediators of Inflammation

Stimulated neutrophils release arachidonic acid (AA) from the plasma membrane. AA itself can attract and stimulate neutrophils, and AA metabolites (eicosanoids) are more important to regulation of inflammation (see entry "▶ Prostaglandins, Leukotrienes, and Related Compounds"). The most important lipid mediator of inflammation among these is leukotriene B4 (Samuelsson et al. 1987). In contrast, lipoxin A4, prostaglandin J2, and the resolvins facilitate resolution of inflammation.

Although the relative amount of cytokines produced by neutrophils is small, the large number of neutrophils in sites of inflammation can provide a concentration of cytokines sufficient to recruit additional neutrophils to the target area. Among the cytokines produced by neutrophils are interleukin-8 (IL-8), IL-12, transforming growth factor (TGF)-beta, and growth-related oncogene-alpha (GRO-a). However, activated neutrophils do not produce IL-1, IL-6, and TNF-alpha, the classical products of macrophages and synovial cells (Scapini et al. 2000).

Neutrophil Extracellular Traps

Spectacular images of microbe-activated neutrophils ejecting nuclear chromatin coated with granule-derived bactericidal peptides and enzymes such as elastase, cathepsin G, and myeloperoxidase were presented in 2004 (Brinkmann et al. 2004). These structures, called neutrophil extracellular traps (NETs) because they entangle bacteria, are efficient killing machines but also appear to participate in the pathogenesis of several autoimmune and inflammatory disorders. Therefore, in addition to serving as a part of the protective innate immune response, NETs may be generated by noninfectious stimuli (e.g., cytokines) and provide molecules that promote autoimmunity and tissue injury in susceptible individuals (Kaplan and Radic 2012). For example, NET-derived material is detected in blood and kidney biopsies from many patients with small vessel vasculitis, and autoantigens recognized by ANCAs (anti-neutrophil cytoplasmic antibodies characteristic of the disease) are found in these NET structures.

Diseases of Diminished Neutrophil Number

Severe congenital neutropenia (Kostmann's syndrome) results from arrest of bone marrow

myelopoiesis. Infants are prone to severe bacterial infections and at risk for malignancies, and mortality is high. Therapy includes antibiotics and granulocyte colony-stimulating factor (Germeshausen et al. 2010). A milder form (benign congenital neutropenia) has been observed, and cyclic neutropenia exhibits transient recurrent neutropenia on a 21-day cycle.

Leukocyte Adhesion Deficiencies (LAD)

Three leukocyte adhesion disorders have been described: LAD1 results from an autosomal defect in the gene encoding for beta2 integrins. Thus, bloodstream neutrophils are unable to adhere to vascular walls and migrate to target areas. Bone marrow transplantation is the only curative therapy. LAD2 results from a defect in a neutrophil ligand that allows neutrophils to roll along the endothelium. In addition to the symptoms associated with LAD1, patients may have mental retardation and short stature. In patients with LAD3, integrin activation is impaired in platelets as well as in PMN, so that patients are at increased risk for bleeding and for infections (McDowall et al. 2003).

Neutrophil Granule Defects

The best known of these disorders is the Chediak-Higashi syndrome, an autosomal recessive defect in the gene for lysosomal transport protein, in which granules fuse to create giant dysfunctional lysosomes. Patients present with partial oculocutaneous albinism, neutropenia, frequent infection, mild bleeding, and neurologic abnormalities (Zurier 1977; Barbosa et al. 1996). Ascorbic acid has been useful treatment, but patients who survive childhood often succumb to a lymphoma-like disease (Boxer et al. 1979).

Oxidase Deficiencies: Chronic Granulomatous Disease (CGD)

CGD is a group of diseases. In each, a genetic defect in a different component of NADPH oxidase results in failure of neutrophils to generate superoxide, impairing intracellular killing and the ability to generate NETS. The accumulation of neutrophils that cannot kill bacteria at a site of infection results in granuloma formation rather than clearance of bacteria and cells (van den Berg et al. 2009).

Cross-References

- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Neutrophils in Endothelial Damage
- ▶ Prostaglandins, Leukotrienes, and Related Compounds
- ▶ Resolution of Inflammation
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Barbosa MD, Nguyen QA, Tcherneve VT, et al. Identification of the beige and Chediak-Higashi syndrome genes. Nature. 1996;382:262–5.

Bokoch GM. Chemoattractant signaling and leukocyte activation. Blood. 1995;86:1649–60.

Borregaard N. Neutrophils from marrow to microbes. Immunity. 2010;33:657–70.

Boxer LA, Albertini DF, Baehner RL, et al. Impaired microtubule assembly and polymorphonuclear leukocyte function in the Chediak-Higashi Syndrome correctable by ascorbic acid. Br J Haematol. 1979;43:207–13.

Brinkmann V, Reichard U, Goosman C, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303:1532–5.

Caron E, Hall A. Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. Science. 1998;282:1717–21.

DeLeo FR, Quinn MT. Assembly of the phagocyte NADPH oxidase: molecular interaction of oxidase proteins. J Leukoc Biol. 1996;60:677–91.

Germeshausen M, Zeidler C, Bolyard AA, et al. Digenic mutations in severe congenital neutropenia. Haematologica. 2010;95:1207–10.

Kaplan MJ, Radic M. Neutrophil extracellular traps: double edged swords of innate immunity. J Immunol. 2012;189:2689–95.

McDowall AA, Inwald D, Leitinger B, et al. A novel form of integrin dysfunction involving beta1, beta2, and beta3 integrins. J Clin Invest. 2003;111:51–60.

Samuelsson B, Dahlen SE, Lindgren JA, et al. Leukotrienes and lipoxins: structure, biosynthesis, and biological effects. Science. 1987;237:1171–6.
Scapini P, Lapinet-Vera JA, Gasperini S, et al. The neutrophil as a cellular source of chemokines. Immunol Rev. 2000;177:195–203.
Scher JU, Abramson SA, Pillinger MH. Neutrophils. In: Firestein GS, Budd RC, Gabriel SE, McInnes IB, editors. Kelley's textbook of rheumatology. 9th ed. Philadelphia: Elsevier/Saunders; 2013. p. 152–69.
Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008;8:349–61.
van den Berg JM, van Koppen E, Ahlin A, et al. Chronic granulomatous disease: the European experience. PLoS One. 2009;4:e5234.
Zurier RB. Lysosomes and dermatology. Dermatology. 1977;16:726–35.

Neutrophils in Endothelial Damage

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Definition

Neutrophils are vital innate immune cells that are indispensable in defending the body against invading pathogens. Neutrophils migrate from the lumen of blood vessels, crossing the endothelial cell barrier in order to reach the infection sites. Neutrophils release a number of enzymes and reactive products such as oxidants while eliminating pathogens. If the products released by the neutrophils are not quickly neutralized, they cause unintended damage to endothelial cells and surrounding tissues, often resulting in pathological conditions such as acute lung injury and arthritis.

Neutrophils

Neutrophils (PMNs) are an integral part of the innate immunity arsenal that protects against invading pathogens such as bacteria but also in "sterile inflammation" (in the absence of pathogens) that occurs as a consequence of hypoxia/reperfusion injury, wound repair, and crystal-induced injury such as in gout when monosodium urate crystals are deposited in joints. PMNs are the first responders to invading pathogens and limit the spread of infection. In normal adult humans, PMNs are the most abundant innate immune cells in blood and are produced in the bone marrow from stem cells. As many as $1-2 \times 10^{11}$ PMNs can be generated per day (Borregaard 2010). PMNs are highly motile phagocytic cells that engulf and degrade bacteria with the help of factors stored in intracellular granules as well as the generation of reactive oxygen species (ROS) via the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) pathway. In addition, recent studies suggest that PMNs are capable of killing bacteria extracellularly by releasing neutrophil extracellular traps (NETs) consisting of extruded chromatin embedded with microbicidal peptides including histones, which can attack and eliminate the entrapped bacteria. Further, there is a growing appreciation that PMNs, when activated, release a number of cytokines and initiate "cross talk" with other cells involved in innate and adaptive immunity, such as dendritic cells and Th17 cells. In turn, this intercellular communication results in recruitment of more PMNs and release of mediators at the site of infection, underscoring the complexity of PMN function (Mantovani et al. 2011).

Under normal circumstances, PMNs are continuously produced by the bone marrow and removed from tissue or blood by macrophages in the spleen, bone marrow, and liver (Bratton and Henson 2011). PMNs have a short life-span (in the order of hours) in the circulation, although recent reports suggest more in the order of a few days (Bratton and Henson 2011). Maintenance of adequate PMN numbers is dependent on the intrinsic ability of aging neutrophils to undergo apoptosis. However, during infections or injury, PMN production can be ramped up by accelerated release of PMNs from the bone marrow. In this context, G-CSF is produced, causing both a rapid expansion of PMNs in the bone marrow and PMN release into the blood.

Endothelial Cells

Far from being an irrelevant bystander, the endothelium serves as barrier between the circulating blood and the extravascular environment and plays an active and dynamic role in facilitating the transport of leukocytes, plasma proteins, and fluids across this barrier while maintaining tissue homeostasis (Komarova and Malik 2010). Endothelial cells maintain blood flow in a non-thrombotic environment by actively inhibiting coagulation. This is achieved by the expression of thrombomodulin on endothelial cell surfaces that binds thrombin and helps in the activation of protein C, a potent anticoagulant. Expression of tissue factor pathway inhibitors that bind to activated clotting factors X and VIIa complexed with the strong procoagulant, tissue factor, prevents downstream effects leading to intravascular clot formation. The expression of heparan sulfate proteoglycans results in inactivation of thrombin, thus preventing clot formation (Pober and Sessa 2007). The endothelium also maintains vascular tone and helps to direct and traffic leukocytes to the appropriate extravascular inflammatory sites. Endothelial cells are relatively impermeable to leukocytes and to various proteins such as albumin and IgG molecules due to tight (containing proteins such as occludin) and adherens junctions (containing proteins such as VE-cadherin and β-catenin) found between adjoining endothelial cells. Nitric oxide (NO; vasodilator) and prostacyclin are also produced by endothelial cells. Together, they inhibit platelet aggregation and adherence of platelets to the endothelial cell membrane. Transport of solutes such as albumin, lipids, insulin, and hormones also occurs with the help of vesicles called caveolae that coalesce with the endothelial cell surface in an energy-dependent process (Komarova and Malik 2010).

Factors that can affect vascular permeability include histamine, bradykinin, thrombin, lipopolysaccharide, TNF-α, and vascular endothelial growth factor (VEGF), to name just a few. These permeability-inducing factors bind to receptors on endothelial cell surfaces and signal through guanine nucleotide-binding proteins (G proteins), leading to actin and myosin polymerization, which results in endothelial cell contraction and reversible disruption of junctions, thereby allowing solutes and fluid to pass into extravascular locales (DiStasi and Ley 2009).

Products of Neutrophils That Are Injurious to the Endothelium

Enzymes and Proteases

Various PMN-associated functions are mediated in part by the contents of the cytoplasmic granules and vesicles. The granules are classified into distinct subsets based on their characteristic contents (Table 1). *Primary* (azurophilic) granules contain antimicrobial proteins including myeloperoxidase (MPO) which forms hypochlorous acid (HOCl) in the presence of H_2O_2 produced by the NADPH oxidase system. In addition, primary granules contain defensins which are small peptides (approximately 3.5 kDa) that are cytotoxic to bacteria by generating pores in the bacterial cell membranes. Primary granules also contain serine proteases that aid in phagocytosis. *Secondary* (specific) granules contain lactoferrin (that has antimicrobial activity), lysozyme, and components of the NADPH oxidase system, facilitating antimicrobial defense. *Tertiary* (gelatinase) granules contain gelatinase and other matrix-degrading enzymes (matrix metalloproteinases, MMPs) and membrane receptors that are involved in extravasation or diapedesis of PMNs from the blood vessel into tissues. Additionally, PMNs also contain secretory vesicles that act as storage organelles for membrane proteins including complement receptors and integrins. Upon mobilization of the PMN, the secretory vesicles merge with the cell membrane, resulting in addition of vesicle receptors to the PMN cell membrane. These membrane proteins enhance attachment to the endothelium in addition to replacing plasma membrane proteins expended during phagocytosis. Secretory vesicles are thought to be mobilized first, followed by tertiary granules, secondary granules, and primary granules, although the exact order may vary depending on circumstances of the inflammatory response. Many of the PMN

Neutrophils in Endothelial Damage, Table 1 A representative list of proteins and enzymes found in various neutrophil granules and vesicles

Primary granules (azurophilic)	Secondary granules (specific)	Tertiary granules (gelatinase)	Secretory vesicles
Myeloperoxidase	Lactoferrin	Gelatinase	Complement receptor-1
Lysozyme	Collagenase	Cytochrome b_{558}	CD11a/CD18 (LFA-1)
Elastase	Gelatinase	β_2-microglobulin	CD11b/CD18 (MAC-1)
Hydrolases	Lysozyme	fMLP receptor	
Defensin	Cytochrome b_{558}		
Phospholipases			
Azurocidin			

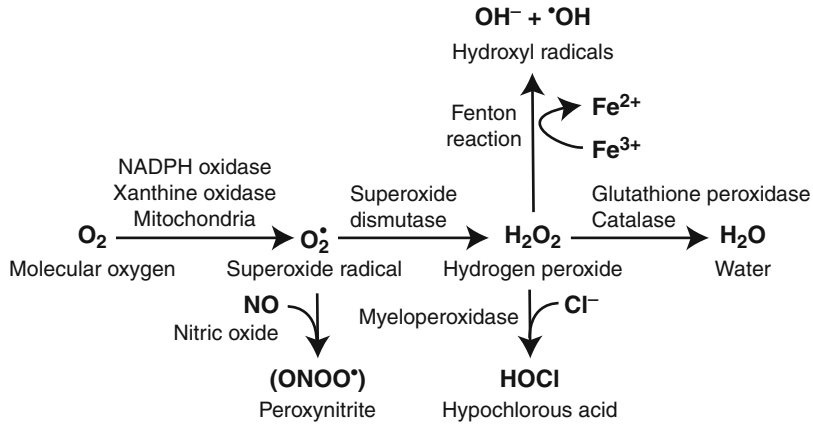
enzymes or proteases such as collagenase (MMP-8), gelatinase (MMP-9), and leukolysin (MMP-25) are stored in an inactive form and are activated by proteolytic cleavage during the process of degranulation. These enzymes participate in breakdown of the extracellular proteins such as laminin, fibronectin, collagen type IV, and heparan sulfate proteoglycans, all linked to the extravasation process of PMNs (Faurschou and Borregaard 2003). Although the release of these granule-derived mediators is a tightly regulated exocytosis process, there occurs unintended collateral damage to the tissues that are infiltrated by the PMNs (Nathan 2006; Borregaard 2010).

Oxidants

PMNs upon activation assemble the potent NADPH oxidase system at their cell surfaces, resulting in the formation of highly reactive oxygen metabolites that help combat pathogens. NADPH oxidase catalyzes the conversion of molecular oxygen (O_2) to superoxide anion (O_2•). NADPH oxidase is one of the main sources of oxidant generation in vivo. NADPH oxidase of PMNs contains a number of subunits, with gp91 phox and p22 phox together constituting the membrane-bound cytochrome b_{558} subunit, along with several cytosolic subunits: p67phox, p47phox, p40phox, and Rac 1 and Rac 2 small Rho guanosine triphosphatases (Babior 2004). When activated, the cytosolic subunits translocate and associate with membrane-bound subunits, leading to the formation of the active enzyme NADPH oxidase. There are seven homologues of gp91 phox that have been found to date. gp91 phox is also referred to as NOX2 gp91 and is found in phagocytic cells and in endothelial cells along with the NOX1, NOX4, and NOX5 isoforms. Although O_2• has a limited half-life, it is quickly converted to H_2O_2 in the presence of superoxide dismutase (SOD) (Fig. 1). MPO catalyzes conversion of H_2O_2 to hypochlorous acid (HOCl) or hypobromous acid (HOBr) in the presence of chloride or bromide. Catalases and peroxidases (such as glutathione peroxidase) convert H_2O_2 into H_2O and O_2, while glutathione peroxidase converts H_2O_2 to H_2O. Iron (Fe^{3+}) is present in endothelial cells and bound to ferritin. It interacts with O_2•, resulting in reduction of Fe^{3+} to Fe^{2+}. Fe^{2+} then interacts with H_2O_2 to form the hydroxyl radical, HO•. Hydroxyl radicals are highly reactive with proteins and lipids. Collectively, these ROS products importantly combat invading pathogens. Recent studies suggest that ROS can subserve several functions, especially in cell signaling. They appear to be not only involved in host defense but also in maintaining homeostasis. ROS can activate signaling molecules such as MAP kinases and transcription factors such as NFκB and regulate gene expression by affecting histone acetylation and deacetylation in the nucleus (Ma 2010). Excessive levels of ROS will lead to tissue damage.

The Inflammasome

PMNs can also detect bacteria and other microbes by germ line-encoded pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) expressed on the cell surface, and nucleotide-binding oligomerization domain-like

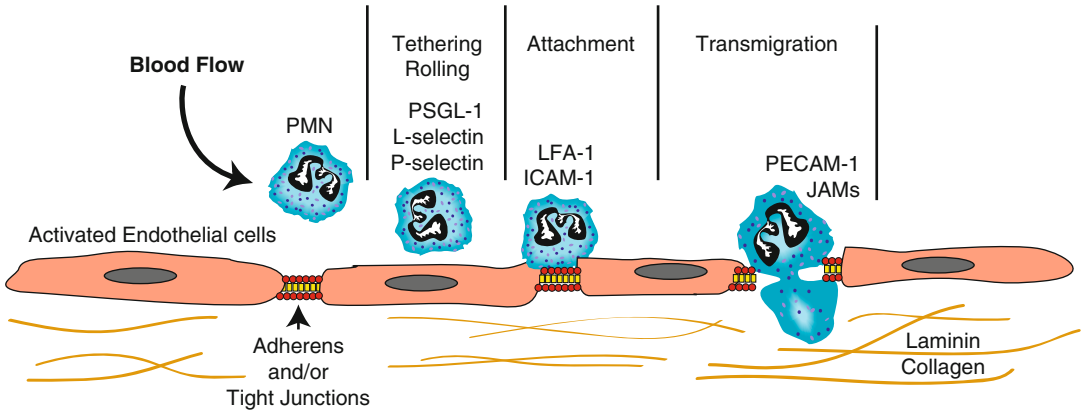


Neutrophils in Endothelial Damage, Fig. 1 Examples of pathways leading to generation of reactive oxygen species (ROS). Superoxide (O_2•) and hydrogen peroxide (H_2O_2) are formed when molecular oxygen (O_2) is reduced. Glutathione peroxidase and catalase catalyze the conversion of H_2O_2 to water (H_2O) and molecular oxygen (O_2). The Fenton reaction (in the presence of Fe^{2+}) leads to the formation of highly unstable hydroxyl radical ($OH^•$) from H_2O_2. Myeloperoxidase converts H_2O_2 to hypochlorous acid (HOCl) in the presence of chloride ions (Cl^-). Peroxynitrite is formed when nitrous oxide (NO) interacts with superoxide

receptors (NLRs) expressed intracellularly. On detecting pathogens in the cytosol, the intracellular PRRs form a multiprotein complex called the "inflammasome," resulting in activation of the cysteine protease, caspase-1. In turn, activated caspase 1 (and perhaps other caspases) promotes the proteolytic cleavage of the inactive precursor forms of IL-1β and IL-18 to active forms, which are then secreted from PMNs. Inflammasomes promote clearance of the infected cells and initiate caspase 1-mediated apoptosis or pyroptosis (Lamkanfi and Dixit 2011). The inflammasome has been linked to diabetes mellitus as well as crystal-induced joint damage (gout) and a variety of other inflammatory responses.

Evidence of Neutrophil-Mediated Endothelial/Tissue Damage

Endothelial cell injury by activated PMNs both in vivo and in vitro is now well known. Endothelial damage in vitro by complement activated PMNs was originally described by Sacks et al. (Sacks et al. 1978). There is now abundant evidence that PMNs in large part mediate endothelial cell/tissue damage in vivo by a wide variety of mechanisms, such as release of damaging oxidants and proteolytic enzymes (Guo and Ward 2002; Gao et al. 2006) when activated PMNs become more adherent to the endothelium and extravasate through the vascular wall into tissues. There is unequivocal evidence in vivo that PMNs contribute to tissue and organ damage due to release of proteases as well as oxidants (ROS, RNS). PMNs contribute to the pathophysiology of many conditions including stroke, rheumatoid arthritis, myocardial infarction, immune vasculitis, inflammatory bowel disease, acute lung injury (ALI), and acute respiratory distress syndrome (ARDS). In patients with ARDS, the number of PMNs present in the bronchoalveolar lavage fluids, as well as presence of complement activation product, component 5a (C5a), correlates with the severity of ARDS (Pittet et al. 1997). Lung edema, disruption of the endothelial and epithelial barrier due to gap and bleb formation, and increased PMN infiltration into the interstitium and bronchoalveolar spaces are associated with release of proinflammatory mediators such as TNF-α and IL-8, causing compromised lung function and air exchange. In systemic inflammation, the presence of inflammatory mediators including C5a, together with IL-8 and TNF-α, is associated with neutrophilia and organ damage.



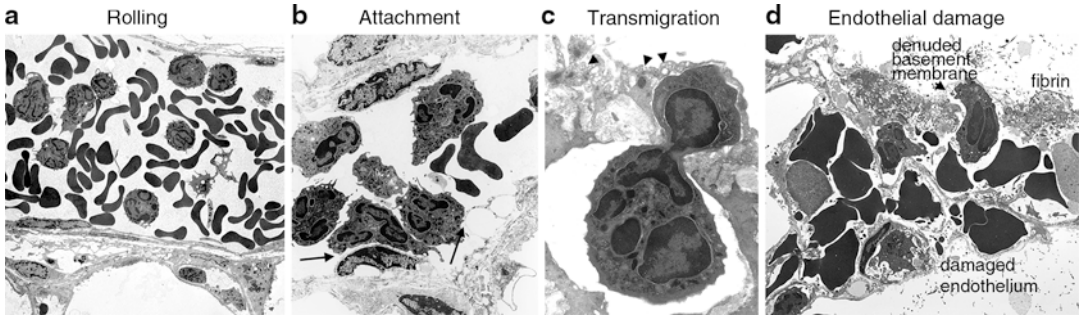
Neutrophils in Endothelial Damage, Fig. 2 Intermittent adherence of PMNs to the endothelium, followed by firm attachment and transmigration. Normally, the tight junctions as well as the adherens junctions maintain integrity of the endothelial cell wall and prevent leakage of water and soluble plasma contents into extra vascular spaces

In a rodent model of acute lung injury, deposition of IgG immune complexes (IgGIC) in the lung leads to complement activation and to lung PMN accumulation, with compromise of the lung endothelial and epithelial barrier functions (Guo and Ward 2002; Gao et al. 2006). When lungs were treated with anti-elastase, lung injury was significantly reduced, further implicating PMNs in inflicting tissue damage and thus participating in the pathogenesis of lung injury (Guo and Ward 2002; Gao et al. 2006).

In the collagen-induced arthritis model (where arthritis is induced by immunization with type II collagen) and the K/BxN arthritis model (arthritis model based on the expression of the T cell receptor transgene KRN and the MHC class II molecule A(g7) where mice develop autoantibodies against glucose-6-phosphate isomerase (GPI)) that closely resemble human arthritis, PMNs play an early role in the development of the disease process. PMNs are the first innate immune cells to infiltrate the joint, persisting in the synovial fluid and in synovial tissues during the course of the disease, further underscoring PMN-mediated tissue/organ damage. In PMN-depleted animals, arthritis fails to develop, while in animals with arthritic joints, systemic depletion of PMNs leads to reversal of the disease manifestations (Wright et al. 2010).

Neutrophil-Endothelial Cell Interaction

One of the first steps involved in the recruitment of PMNs to the site of inflammation is known as "rolling." It involves the dynamic interaction of the PMNs with the endothelium with the help of transmembrane adhesion proteins and their cognate ligands (Figs. 2 and 3). These include selectins, integrins, and immunoglobulin superfamily members such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). P-selectin stored in Weibel-Palade granules in endothelial cells and E-selectin produced de novo in endothelial cells, appearing on apical surfaces, bind to P-selectin glycoprotein ligand-1 (PSGL-1), E-selectin ligand (ESGL), and CD44 that are expressed by PMNs (Phillipson and Kubes 2011). This results in transient binding of PMNs to the endothelial surfaces (rolling). ICAM-1 present on the endothelium interacts with the β_2-integrins CD11a/CD18 (LFA-1), while VCAM-1 interacts with α_4-integrins ($\alpha_4\beta_1$, $\alpha_4\beta_7$), resulting in slowing of rolling PMNs and their firm adhesion to the endothelium followed by migration into the tissue with the help of endothelial membrane proteins: platelet endothelial cell adhesion molecule-1 (PECAM-1), endothelial cell-selective adhesion molecule-1 (ESAM-1), and CD99 and the β-integrin CD11b/CD18 (Mac 1) on PMNs.



Neutrophils in Endothelial Damage, Fig. 3 Transmission electron micrographs of neutrophil-endothelial cell interactions in rat lungs following acute lung injury. Transient binding of PMNs to the endothelial cell (frame **a**, rolling) leads to firm attachment of PMNs to the endothelium (*arrows* in frame **b**), followed by transmigration (frame **c**, *small arrows* indicate the presence of caveolae) into extravascular sites. This process sometimes results in endothelial damage (frame **d**), resulting in denuded basement membrane and fibrin deposits (fixation by glutaraldehyde, staining with uranyl acetate and lead citrate; **a**, X1750; **b** and **d**, X2150; **c**, 3550)

Junction adhesion molecule c (JAM-c) present at the junctions of inter-endothelial contacts prevents reverse migration into the blood vessel lumen (Phillipson and Kubes 2011). Activated PMNs can extravasate into tissues or interstitium by modifying junctional proteins (via phosphorylation) mediated by PMN-generated products including ROS.

Animal models have been helpful in confirming the roles of various adhesion molecules in mediating PMN recruitment. In the IgG model of lung injury, PMN recruitment and consequent development of lung injury were greatly reduced when L-, P-, or E-selectins were blocked using neutralizing antibodies. Similarly, blocking of ICAM-1 with antibodies caused significant reductions in vascular permeability and MPO levels in tissues (a marker for PMN degranulation) (Guo and Ward 2002; Gao et al. 2006).

During the resolution phase of the inflammation, PMNs undergo apoptosis resulting in signals that alert local macrophages for quick removal of PMNs before these cells release their cytoplasmic contents into the milieu. If PMNs are not cleared and their products of degranulation are not neutralized by the endothelium, tissue injury can proceed. Products of PMNs such as MPO can cause endothelial cell blebbing and endothelial cell detachment, while proteinase 3 present in primary granules can induce apoptosis (Tesfamariam and DeFelice 2007). Elastase and cathepsin increase vascular permeability. The activity of these proteinases and MMPs is regulated by endogenous proteinase inhibitors such as α_1-proteinase inhibitor, α_2-macroglobulin, secretory leukocyte protease inhibitor (SLP1), and tissue inhibitor of metalloproteinase-1 (TIMP-1) (Gao et al. 2006) present in the endothelium.

Further, there are several sources of oxidants in the endothelium. NADPH oxidase, xanthine oxidase, and mitochondria can each contribute to oxidant formation. Endothelial cells produce nitric oxide (NO) by nitric oxide synthase (NOS), employing L-arginine. These cells contain eNOS or NOS3 isoforms that are calcium dependent. NO can interact with oxidants to form peroxynitrite ($ONOO^-$) that is cell toxic (Fig. 1). Additionally, there are several enzymatic and nonenzymatic antioxidants that can neutralize ROS (Forstermann 2010). For instance, beta-carotene and vitamins E and C act as nonenzymatic antioxidants, while superoxide dismutase, catalase, thioredoxin, hemeoxygenases, and glutathione peroxidase act as enzymatic antioxidants. Excessive oxidant products lead to tissue damage or destruction by oxidant-mediated modifications of cellular

proteins, carbohydrates, lipids, and DNA leading to their altered function (Lum and Roebuck 2001). For example, changes in permeability can be ascribed to oxidation of lipids in the cell membrane. Superoxide generation is thought to compromise the endothelial barrier to permeability.

Interestingly, repeated infections are found in patients with either inadequate numbers (neutropenia) or inadequate functional capacity of PMNs. Neutropenia can occur due to congenital defects, acquired autoimmunity, or bone marrow failure brought about by metastatic tumor, hematopoietic cancers, or chemotherapy. Leukocyte adhesion deficiencies (LADs), chronic granulomatous disease (CGD), and specific granule deficiency are the result of defective functional components of the NADPH oxidase (CGD), defective adhesion molecules (LAD), and lack of production of granule proteins (SGD).

Conclusion

PMNs play an important role in host defenses. However, in the deployment of the powerful arsenal that PMNs possess in combating invading pathogens and local "sterile" (uninfected) inflammation, unintended consequences can occur, leading to endothelial/tissue damage. Some therapeutic interventions such as corticosteroids can minimize tissue damage while still facilitating the ability of PMNs to eliminate harmful organisms and also produce mediators of injury repair, as occurs in noninfected tissues/organs damaged by ischemic conditions.

Cross-References

- ▶ Animal Models in Rheumatoid Arthritis
- ▶ Antioxidants
- ▶ Cell Adhesion Molecules
- ▶ Mechanisms of Endothelial Activation
- ▶ Nitric Oxide
- ▶ Normal Immune Function and Barrier: Defensins
- ▶ Normal Immune Function and Barrier: Epithelial Barrier
- ▶ Resolution of Inflammation

References

Babior BM. NADPH oxidase. Curr Opin Immunol. 2004;16:42–7.

Borregaard N. Neutrophils, from marrow to microbes. Immunity. 2010;33:657–70.

Bratton DL, Henson PM. Neutrophil clearance: when the party is over, clean-up begins. Trends Immunol. 2011;32:350–7.

DiStasi MR, Ley K. Opening the flood-gates: how neutrophil-endothelial interactions regulate permeability. Trends Immunol. 2009;30:547–56.

Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. Microbes Infect. 2003;5:1317–27.

Forstermann U. Nitric oxide and oxidative stress in vascular disease. Pflugers Arch. 2010;459:923–39.

Gao H, Neff T, Ward PA. Regulation of lung inflammation in the model of IgG immune-complex injury. Annu Rev Pathol. 2006;1:215–42.

Guo RF, Ward PA. Mediators and regulation of neutrophil accumulation in inflammatory responses in lung: insights from the IgG immune complex model. Free Radic Biol Med. 2002;33:303–10.

Komarova Y, Malik AB. Regulation of endothelial permeability via paracellular and transcellular transport pathways. Annu Rev Physiol. 2010;72:463–93.

Lamkanfi M, Dixit VM. Modulation of inflammasome pathways by bacterial and viral pathogens. J Immunol. 2011;187:597–602.

Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. Am J Physiol Cell Physiol. 2001;280:C719–741.

Ma Q. Transcriptional responses to oxidative stress: pathological and toxicological implications. Pharmacol Ther. 2010;125:376–93.

Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol. 2011;11:519–31.

Nathan C. Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol. 2006;6:173–82.

Phillipson M, Kubes P. The neutrophil in vascular inflammation. Nat Med. 2011;17:1381–90.

Pittet JF, Mackersie RC, Martin TR, Matthay MA. Biological markers of acute lung injury: prognostic and pathogenetic significance. Am J Respir Crit Care Med. 1997;155:1187–205.

Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol. 2007;7:803–15.

Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacob HS. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. J Clin Invest. 1978;61:1161–7.
Tesfamariam B, DeFelice AF. Endothelial injury in the initiation and progression of vascular disorders. Vascul Pharmacol. 2007;46:229–37.
Wright HL, Moots RJ, Bucknall RC, Edwards SW. Neutrophil function in inflammation and inflammatory diseases. Rheumatology. 2010;49:1618–31 (Oxford).

NF-κB

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Synonyms

c-Rel; NF-κB2 (p52 and its precursor p100); Nuclear factor κB; NF-κB1 (p50 and its precursor p105); RelA (p65); RelB

Definition

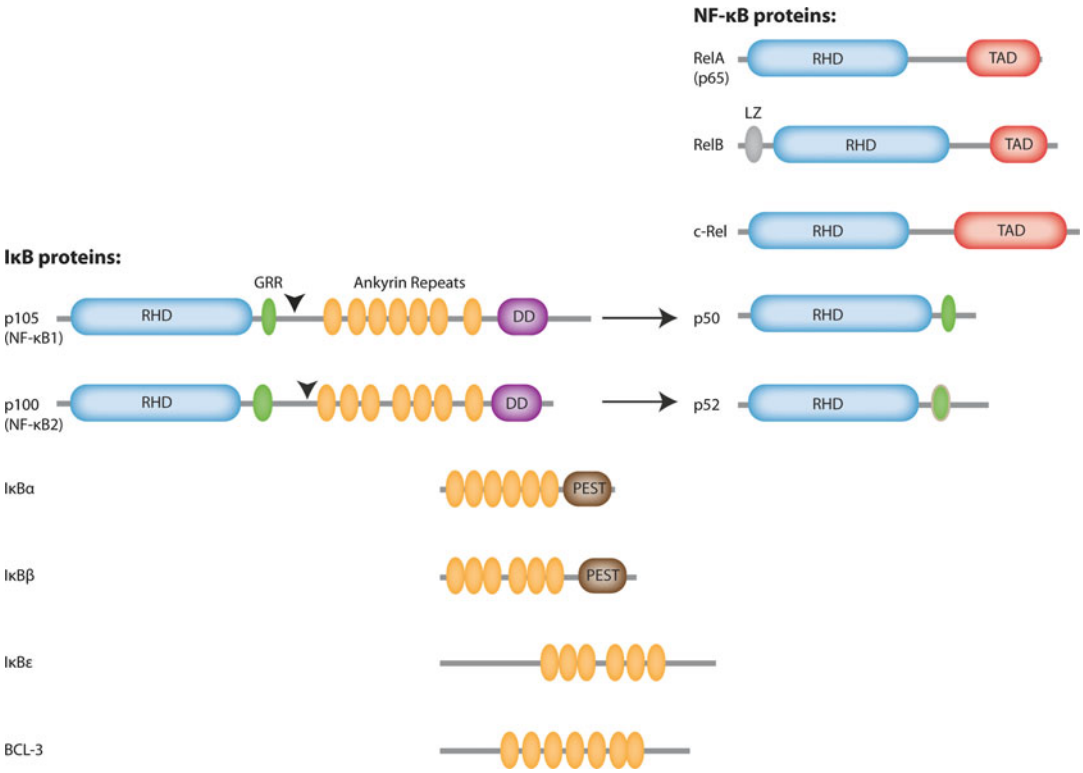
NF-κB is a dimeric transcription factor complex active throughout all cell types. It is present in the cytoplasm in an inactive state, can rapidly be activated in response to various stimuli, and subsequently translocates to the nucleus. NF-κB has a pivotal role in the control of proliferation and apoptosis. Increased NF-κB activity has been shown to contribute to tumorigenesis. Furthermore, it has important functions in both innate and adaptive immune responses.

The NF-κB Transcription Factors

The NF-κB activity was identified more than 25 years ago by defining a nuclear factor that binds DNA sequences in the immunoglobulin κ-light chain region in activated B cells (Sen and Baltimore 1986). This factor was shown to be preexisting in an inactive state in cells and was readily activated by a variety of stimuli. There are five NF-κB family members, namely, RelA (p65), RelB, c-Rel, p50, and p52 (Fig. 1). In their amino-terminus, they have a common Rel-homology domain (RHD), which confers DNA binding and contains a nuclear localization signal (NLS) (Fig. 1) (Ghosh et al. 2012). Only three members, RelA, RelB and c-Rel, have a transactivation domain (TAD) in their carboxy-terminal part and are directly able to induce transcription. p50 and p52 are produced from precursors p105 and p100, respectively (Fig. 1), and are processed by the proteasome. NF-κB transcription factors function as homo- or heterodimers. They interact via the RHD region in the amino-terminus and bind to DNA in a sequence-specific manner (Ghosh et al. 2012). Homodimers of either p50 or p52 can act as transcriptional repressors either by competing for DNA binding or by recruiting histone deacetylases (HDACs) leading to a closed chromatin formation. Most of the other NF-κB dimers activate gene transcription by interaction with the transcription machinery or via recruitment of histone acetylases (HAT), which open up the chromatin and favor the assembly of the preinitiation complex (PIC) on promoter regions (Hayden and Ghosh 2012).

Regulation of NF-κB Activity

The NF-κB transcription factors are kept inactive through the binding of cytoplasmic inhibitors of NF-κB (IκBs), which include the small IκBα, IκBβ, and IκBε proteins, as well as the larger cytoplasmic precursor proteins of p50 and p52, termed p105 and p100, respectively (Fig. 1) (Hinz et al. 2012). The IκBs contain ankyrin repeats through which they bind Rel-homology domains (Fig. 2). Small IκB proteins can bind NF-κB dimers and mask their NLS and additionally contain nuclear export signals (NES). Both mechanisms confer effective cytoplasmic sequestration of the complex, preventing NF-κB from binding to DNA. Upon activation, signal-induced proteolysis of IκBs releases the bound transcription factors that then translocate to the

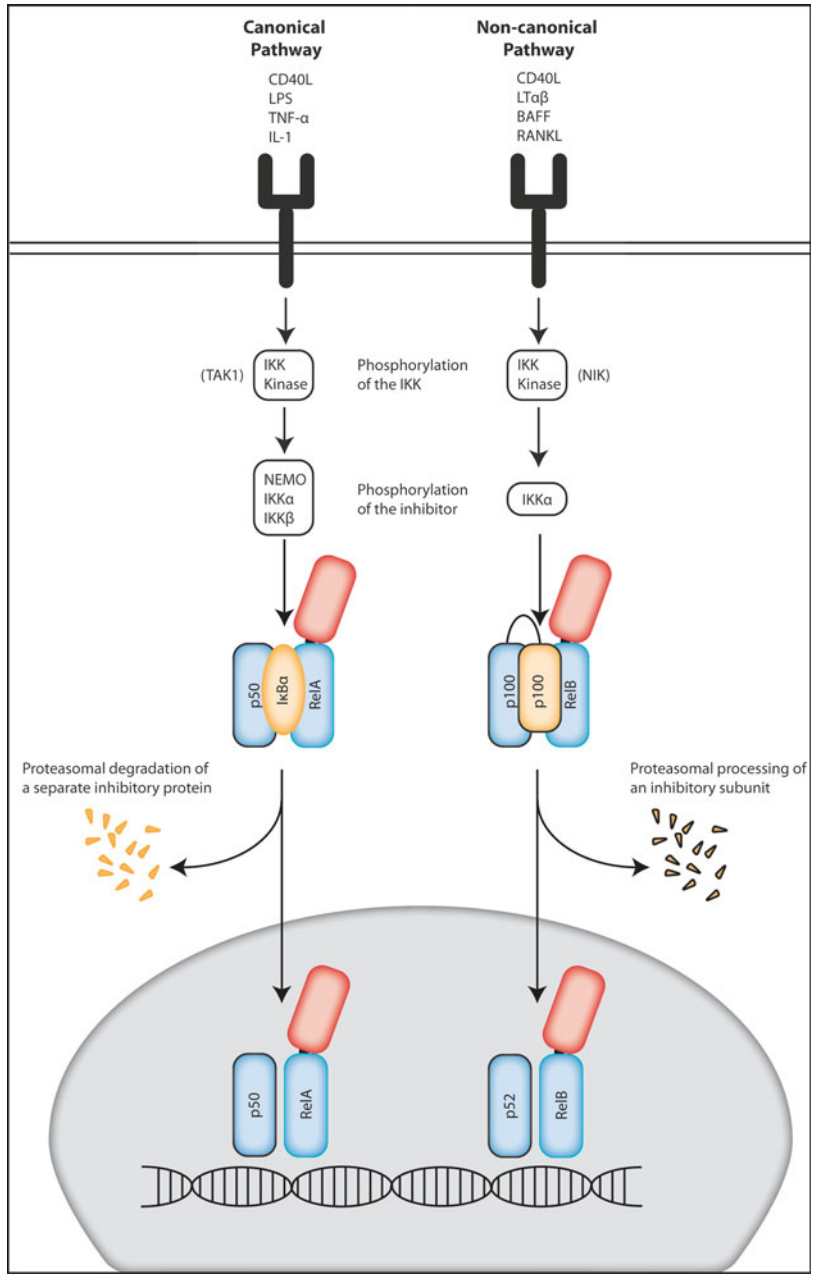


NF-κB, Fig. 1 NF-κB and IκB family members. The domain structure of the human NF-κB and IκB proteins is schematically displayed. *Arrowheads* at p105 and p100 indicate the sites of proteasomal processing. *RHD* Rel-homology domain, *TAD* transactivation domain, *LZ* leucine zipper, *DD* death domain, *GRR* glycine rich region, *PEST* domain rich in proline (P), glutamate (E), serine (S), and threonine (T), *Bcl-3* B cell lymphoma-3

nucleus and activate transcription (Fig. 2) (Ghosh et al. 2012). Not all IκBs are cytoplasmic; the Bcl-3 protein, for example, is an atypical nuclear IκB (Fig. 1), which can bind to p50 or p52 homodimers to form ternary complexes on DNA in vitro (Bours et al. 1993; Fujita et al. 1993). Bcl-3 controls the expression of certain target genes; however, at the moment, it is unclear through which molecular mechanism Bcl-3 actually exerts this function (Vallabhapurapu and Karin 2009). The ankyrin repeats that form an IκB are also found in the carboxy-terminal part of the NF-κB members p50 and p52, which are produced as the precursors p105 and p100 (Fig. 1) (Hinz et al. 2012). The IκB-containing carboxy-terminus is removed by proteasomal processing. The p105 protein is constitutively and co-translationally processed to p50, whereas p100 is processed to p52 in an inducible manner (see below). Transcriptional NF-κB activity is typically achieved by activation of p50/p65 and p50/c-Rel heterodimers or of p52/RelB through the pathways of canonical and noncanonical NF-κB activation, respectively.

Canonical and Noncanonical NF-κB Signaling

Nuclear translocation and transcriptional activity of NF-κB can be induced in all cell types and through a variety of stimuli. NF-κB activating agents typically induce the catalytic activity of the IκB kinases IKKα or IKKβ. In the canonical NF-κB signal transduction cascade (Fig. 2, left panel), transcriptional activation of p50/p65 or p50/c-Rel heterodimers is induced. This occurs, for example, in response to stimulation by



NF-κB, Fig. 2 The canonical and noncanonical NF-κB pathways. Two pathways are triggered by different stimuli and differ mainly in the mechanism of inhibition: the canonical pathway uses a separate inhibitor protein (IκB), and the noncanonical pathway uses a precursor protein (p100), which contains both an inhibitory domain and an RHD domain (for details, see text). The RHD responsible for DNA binding is shown in *blue*, and the TAD is shown in *red*. In the canonical pathway, IκB is marked for proteasomal degradation by the IKKα/IKKβ/NEMO complex, whereas in the noncanonical pathway, phosphorylation of p100 is conducted by IKKα alone. At present, the best candidates for the IKK upstream kinases are NIK (NF-κB-inducing kinase) in the noncanonical pathway and TAK1 (TGF-β activated kinase 1) in the canonical pathway

TNF-α causing the predominant activation of IKKβ within a heterotrimeric complex with IKKα and the regulatory subunit IKKγ, also called NEMO (NF-κB essential modulator) (Fig. 2), and results in the phosphorylation of the small IκB protein IκBα at serines 32 and 36 for the human protein (Vallabhapurapu and Karin 2009). These phosphorylation marks are then recognized by the phosphorylation-specific SCF^{IkB} E3 ubiquitin ligase complex. This complex confers phosphorylation-dependent polyubiquitination at lysine 19 by building Lys-48-linked ubiquitin chains. Lys-48-linked polyubiquitination triggers proteasomal degradation of the IκBα protein and frees the prototypic NF-κB heterodimer p50/p65 (Fig. 2) (Hinz et al. 2012). Importantly, several

genes that encode for IκBs, for example, *IκBα*, *p105*, and *p100*, are themselves induced by NF-κB transcription factors. Thereby, a negative feedback loop ensures the rapid inactivation of NF-κB gene activation after cellular stimulation. In fact, the small IκBα protein translocates to the nucleus and terminates NF-κB activation by binding to NF-κB transcription factors (Ghosh et al. 2012). Canonical NF-κB signaling includes the release of cytoplasmic NF-κB heterodimers or homodimers that have been sequestered by small IκBs like IκBα, IκBβ, or IκBε. These small IκBs contain similar motifs for IKK-dependent phosphorylation and are also recognized by the same E3 ligase for phosphorylation-dependent polyubiquitination. They are degraded with different kinetics and have different binding preferences for certain NF-κB hetero- or homodimers (Vallabhapurapu and Karin 2009). Canonical NF-κB signaling not only releases NF-κB dimers from small IκBs but also mobilizes p50 homodimers through complete degradation of p105 (Heissmeyer et al. 1999, 2001). This degradation of p105 is different from the co-translational partial degradation of p105 to p50 but similarly liberates p105-associated Rel proteins and thereby markedly increases the abundance of nuclear p50 homodimers (Beinke and Ley 2004). These homodimers lack transactivation potential and have therefore been involved in transcriptional repression of target genes. However, p50 homodimers may also transactivate when bound to DNA in ternary complexes with the nuclear IκB Bcl-3 (Fujita et al. 1993).

The p105 protein can either be processed to p50 or completely degraded after stimulation and is part of the canonical NF-κB signaling pathway. In contrast, p100 is partially degraded to p52 by the proteasome, and its signal-induced processing has been termed noncanonical or alternative NF-κB signaling (Fig. 2, right panel). The p100 protein has a binding preference for RelB, and its partial degradation thereby releases p52/RelB heterodimers. The induced processing of p100 to p52 is independent from IKKβ and IKKγ but depends on the kinase activity of IKKα (Fig. 2) (Hayden and Ghosh 2012). Similar to canonical NF-κB signaling, the phosphorylation marks trigger polyubiquitination due to recognition by the SCF^{IkB} E3 ubiquitin ligase complex (Vallabhapurapu and Karin 2009). However, noncanonical NF-κB signaling occurs in response to a different set of receptors and requires different upstream signal transduction than canonical NF-κB signaling (Fig. 2). In general, the canonical NF-κB pathway is triggered by pro-inflammatory signals like TNF-α, IL-1, and LPS. The noncanonical NF-κB pathway reacts to stimuli like lymphotoxin (LT), BAFF (B cell activating factor), and RANKL (receptor activator of NF-κB ligand) (Beinke and Ley 2004). CD40L is able to activate both pathways.

The signal transduction cascades from the receptors of noncanonical or canonical NF-κB signal transduction involve multiple steps towards the activation of the IKK complex but ultimately lead to the activation of a kinase that stimulates the activity of IκB kinases. Activation of TAK1 (TGF-β activated kinase 1) triggers predominantly IKKβ-dependent canonical signaling (Vallabhapurapu and Karin 2009), whereas activation of the NF-κB-inducing kinase (NIK) triggers predominantly IKKα-dependent noncanonical signaling (Fig. 2) (Sun 2012). Both cascades somehow use the ubiquitin system. They either activate the ubiquitin recognizing scaffold protein NEMO/IKKγ and recruit the complex to the receptor during canonical signaling or ubiquitinate and degrade NIK constitutively but stabilize it in an inducible fashion for noncanonical NF-κB activation. Despite differential activation of heterodimers by the noncanonical and canonical activation pathways and the potential to control a very specific set of target genes, there is extensive crosstalk and cross-regulation within both pathways. Therefore, only certain NF-κB-dependent phenotypes exhibit strict selectivity for one of the pathways. Besides, the potential regulation of differential target genes by both pathways differs in their activation kinetics. It is likely that the rather fast induction and rapid termination in the canonical pathway compared to the rather slow and more persistent activation in the noncanonical pathway accounts for the importance of both pathways.

NF-κB in the Immune System

NF-κB family members are involved in the regulation of numerous biological processes in all cell types. NF-κB controls cell proliferation and survival and thereby influences developmental processes, but it is at the same time essential for many forms of cellular activation and cell communication (Gerondakis and Siebenlist 2010).

In the innate immune response, the encounter of pathogen triggers Toll-like receptors (TLR) or Nod-like receptors (NLR) that activate NF-κB. In this first line of defense, NF-κB induces and amplifies the production of inflammatory cytokines by innate immune cells. In addition, NF-κB drives the expression of antimicrobial peptides and the production of nitrogen species and plays a major role in increasing the life span and activation of normally very short-lived innate immune cells, such as dendritic cells and neutrophils (Hayden and Ghosh 2011).

Activation of NF-κB in endothelial cells at the site of infection induces upregulation of adhesion molecules that lead to the recruitment and extravasation of leukocytes from the circulation into the inflamed tissue (Hayden and Ghosh 2011). Recruitment of leukocytes into the inflamed tissue enables activated effector lymphocytes to deploy their defense mechanisms in adaptive immunity, in which NF-κB, again, plays a pivotal role.

In the adaptive immune response, NF-κB is activated downstream of antigen and costimulatory receptors and subsequently regulates the maintenance, activation, proliferation, and effector functions of B and T lymphocytes. During activation, T lymphocytes are especially dependent on NF-κB signaling. Aside from their protection from apoptosis by NF-κB, they depend on the autocrine or paracrine stimulation by IL-2 (Hayden and Ghosh 2011). The *IL-2* gene locus is activated by T cell receptor (TCR)- and costimulation-induced NFAT and AP-1 transcription factors but is critically controlled by multiple NF-κB members. The locus is believed to be repressed by the highly abundant p50 homodimers in naive T cells but is then activated after TCR/CD28 stimulation by induced p50/c-Rel and p50/p65 dimers, and c-Rel has been shown to initiate chromatin remodeling of that locus. In differentiated T helper (Th) 1 cells, p65 transactivates *Il-2* gene transcription but is removed from this locus by the transcription factor T-bet (Hwang et al. 2005). This renders Th1 cells dependent on the cytokines IFN-γ and IL-12, the main cytokines that skew primed T cells towards the Th1 lineage. NF-κB signaling also participates in CD4 T helper cell differentiation, and several members contribute to distinct differentiation pathways. For example, p50 and Bcl-3 are essential for Th2 differentiation, whereas RelB and c-Rel are required for the Th1 phenotype (Hayden and Ghosh 2011). Moreover, the noncanonical pathway is critical for the formation of Th17 cells (Sun 2012). The NF-κB pathway influences not only the biology of mature lymphocytes but also their development (Vallabhapurapu and Karin 2009). During thymocyte differentiation, NF-κB supports several developmental stages of conventional and regulatory T cells. There is no influence on NKT development, but in the periphery, their terminal maturation is also under control of the NF-κB pathway. NK cells are exceptional, as uncontrolled NF-κB activity abrogates their development.

A similar importance is evident in the B cell lineage. NF-κB signaling controls B cell development in the bone marrow and regulates virtually all stages in the life of B lymphocytes, where the canonical pathway plays a prominent role. In B cells, NF-κB can be activated through several signal transduction pathways: through the B cell receptor (BCR) itself, through TNF receptor family members like CD40 and the BAFF receptor, or through the TLRs (Kaileh and Sen 2012). The BCR induces the canonical pathway in a transient manner, in contrast to CD40, which activates the canonical and the noncanonical pathways persistently (Kaileh and Sen 2012). Canonical NF-κB signaling delivers a survival signal for early B cell development in the bone marrow as well as for mature B cells in the periphery. In contrast, the noncanonical pathway activated by the BAFF receptor mainly contributes to mature B cell survival but exerts this effect in combination with

BCR signals. Furthermore, the canonical NF-κB pathway is required for B cell activation, class switch recombination, and both T-dependent and T-independent B cell responses (Hayden and Ghosh 2011). The noncanonical NF-κB pathway has two unique functions in B cells: it is essential for the development of marginal zone B cells in the spleen (Vallabhapurapu and Karin 2009) and is responsible for the induction of ICOSL expression on B cells (Hu et al. 2011). This supports the generation of T follicular helper (T_{FH}) cells, which provide B cell help in the germinal center reaction.

Apart from immune cell-intrinsic effects, essential control of lymphocyte development and function is exerted through NF-κB activity in non-hematopoietic cells. For example, thymocyte selection is controlled by NF-κB activity in non-hematopoietic stroma cells such as medullary thymic epithelial cells (mTEC). The NF-κB pathway also contributes to the development and spatial organization of secondary lymphoid organs (Sun 2012). The microarchitecture is essential for coordinated lymphocyte interactions and enables a productive adaptive immune response. The regulation of lymphoid organogenesis is a unique characteristic of the noncanonical NF-κB pathway. RANK signaling stimulates hematopoietic cells to express high levels of $LT\alpha_1\beta_2$ on the surface (Hayden and Ghosh 2011). This triggers the LTβR on stromal cells and leads to the production of chemokines and cell adhesion molecules, which guide lymphocytes during recruitment and distinct zonal segregation within secondary lymphoid organs. The analysis of mice deficient in signaling components of the noncanonical NF-κB pathway underlines their importance, as these mice exhibit severe defects in lymphoid organogenesis ranging from disorganized splenic architecture to a complete lack of lymph nodes and Peyer's patches.

NF-κB in Cancer

The importance of NF-κB for leukemias and lymphomas initially became evident through the apparent sequence homology between the transforming retroviral protein v-Rel and its cellular counterpart c-Rel (Gilmore 2003). Furthermore, chromosomal translocations that removed the IκB part of p100 and allowed expression of a truncated NF-κB2 (i.e., mostly the p52 part of p100) were found in B and T cell lymphomas (Neri et al. 1991). Since then, a number of studies have firmly established constitutive NF-κB activity in tumor lines or tumor samples isolated from patients with acute lymphocyte leukemia, multiple myeloma, chronic myelogenous leukemia, myelodysplastic syndromes, Hodgkin's lymphoma, activated B cell-like diffuse large B cell lymphoma (ABC-DLBCL), or mucosa-associated lymphoid tissue (MALT) lymphoma (Didonato et al. 2012). In addition, increased NF-κB activity was determined in the majority of human cancers, including colon, gastric, pancreatic, ovarian, hepatocellular, breast, head and neck carcinomas, or melanoma (Didonato et al. 2012).

A major advantage that arises from constitutive NF-κB activity for tumor cells or for cells that undergo malignant transformation is the effective protection from apoptosis. This antiapoptotic effect of NF-κB became obvious in p65-deficient mouse embryos. These embryos die in utero due to TNF-induced hepatocyte apoptosis, as they lack the normal and similarly TNF-stimulation-induced protection against apoptosis by NF-κB. The pronounced antiapoptotic effect of NF-κB is mediated, for example, through its transcriptional antiapoptotic targets *BCL2* and *BCLXL* (Vallabhapurapu and Karin 2009). Another critical function of constitutive NF-κB in malignancies is the induction of cell proliferation, which is achieved through upregulation, for example, of *cyclin D1*, *cyclin D2*, and *c-Myc* as well as *c-Myb* target genes (Vallabhapurapu and Karin 2009). A number of lymphoid malignancies have been shown to depend on NF-κB, making this pathway attractive for the development of pharmacological inhibitors (Vallabhapurapu and Karin 2009). In addition, several lymphoid cancers have been found to arise from specific mutations that either activate NF-κB signal

transduction components or inactivate inhibitory cellular regulators of NF-κB (Staudt 2010). In the absence of somatic mutations that could activate this pathway in solid malignancies, NF-κB is nevertheless induced by pro-inflammatory stimuli in the tumor microenvironment or in preceding chronic inflammatory diseases that later on give rise to tumor formation. A recent experimental focus has investigated the connection between inflammation and tumor formation and has involved NF-κB in several cell types. NF-κB is critical in these settings, but it has also been shown to have positive as well as negative effects in different mouse models of tumorigenesis (Didonato et al. 2012).

Conclusion

NF-κB is a family of transcription factors that contain a Rel-homology domain. These Rel proteins form hetero- or homodimers that are present in all cells. NF-κB is normally inactive since the transcription factors are sequestered in the cytoplasm by members of the IκB family. A variety of signals induce a transduction cascade that rapidly causes the proteolysis of IκBs and releases NF-κB dimers from inhibition. Two different NF-κB induction pathways serve overlapping but also unique functions: the canonical pathway is fast but transient, whereas the noncanonical pathway is slower but long-lasting. Stimuli and inducers of canonical and noncanonical activation rapidly induce NF-κB to translocate into the nucleus, where it activates the expression of target genes. Prominent among them are cytokine genes that have pro-inflammatory effects, mediate cell proliferation, or inhibit apoptosis. After NF-κB is induced, it also upregulates target genes from the NF-κB family or from the functionally linked IκB family, leading into positive or negative feedback loops. The biological roles of the NF-κB pathway are numerous and diverse. It plays important roles in the development and homeostasis of innate and adaptive immune cells. In addition, NF-κB is essential for T and B cell activation by their antigen receptors and constitutes the major pathway downstream of receptors that sense pathogen-associated molecular patterns. The breakdown of the tight control of the NF-κB pathway has a well-documented role in inflammation and tumorigenesis.

Cross-References

- ▶ B7 and CD28 Families
- ▶ Bcl-2 Family Members and Lymphocyte Homeostasis
- ▶ CD40
- ▶ Chemokines
- ▶ Cytotoxic T Lymphocytes
- ▶ Normal Immune Function and Barrier: Defensins
- ▶ Nuclear Factor of Activated T Cells (NFAT)
- ▶ PI3K
- ▶ TGF-β
- ▶ Tregs in the Liver

References

Beinke S, Ley SC. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. Biochem J. 2004;382(Pt 2):393–409.

Bours V, Franzoso G, Azarenko V, Park S, Kanno T, Brown K, et al. The oncoprotein Bcl-3 directly transactivates through kappa B motifs via association with DNA-binding p50B homodimers. Cell. 1993;72(5):729–39.

Didonato JA, Mercurio F, Karin M. NF-kappaB and the link between inflammation and cancer. Immunol Rev. 2012;246(1):379–400.

Fujita T, Nolan GP, Liou HC, Scott ML, Baltimore D. The candidate proto-oncogene bcl-3 encodes a transcriptional coactivator that activates through NF-kappa B p50 homodimers. Genes Dev. 1993; 7(7B):1354–63.

Gerondakis S, Siebenlist U. Roles of the NF-kappaB pathway in lymphocyte development and function. Cold Spring Harb Perspect Biol. 2010;2(5):a000182.

Ghosh G, Wang VY, Huang DB, Fusco A. NF-kappaB regulation: lessons from structures. Immunol Rev. 2012;246(1):36–58.

Gilmore TD. The Re1/NF-kappa B/I kappa B signal transduction pathway and cancer. Cancer Treat Res. 2003;115:241–65.

Hayden MS, Ghosh S. NF-kappaB in immunobiology. Cell Res. 2011;21(2):223–44.

Hayden MS, Ghosh S. NF-kappaB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev. 2012;26(3):203–34.

Heissmeyer V, Krappmann D, Wulczyn FG, Scheidereit C. NF-kappaB p105 is a target of IkappaB kinases and controls signal induction of Bcl-3-p50 complexes. EMBO J. 1999;18(17):4766–78.
Heissmeyer V, Krappmann D, Hatada EN, Scheidereit C. Shared pathways of IkappaB kinase-induced SCF (betaTrCP)-mediated ubiquitination and degradation for the NF-kappaB precursor p105 and IkappaBalpha. Mol Cell Biol. 2001;21(4):1024–35.
Hinz M, Arslan SC, Scheidereit C. It takes two to tango: IkappaBs, the multifunctional partners of NF-kappaB. Immunol Rev. 2012;246(1):59–76.
Hu H, Wu X, Jin W, Chang M, Cheng X, Sun SC. Noncanonical NF-kappaB regulates inducible costimulator (ICOS) ligand expression and T follicular helper cell development. Proc Natl Acad Sci USA. 2011;108(31):12827–32.
Hwang ES, Hong JH, Glimcher LH. IL-2 production in developing Th1 cells is regulated by heterodimerization of RelA and T-bet and requires T-bet serine residue 508. J Exp Med. 2005;202(9):1289–300.
Kaileh M, Sen R. NF-kappaB function in B lymphocytes. Immunol Rev. 2012;246(1):254–71.
Neri A, Chang CC, Lombardi L, Salina M, Corradini P, Maiolo AT, et al. B cell lymphoma-associated chromosomal translocation involves candidate oncogene lyt-10, homologous to NF-kappa B p50. Cell. 1991;67(6):1075–87.
Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell. 1986;46(5):705–16.
Staudt LM. Oncogenic activation of NF-kappaB. Cold Spring Harb Perspect Biol. 2010;2(6):a000109.
Sun SC. The noncanonical NF-kappaB pathway. Immunol Rev. 2012;246(1):125–40.
Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol. 2009;27:693–733.

Nitric Oxide

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Synonyms

Endothelium-derived relaxing factor (EDRF); Nitrogen monoxide; Nitrogen oxide; NO; Reactive nitrogen species

Definition

Nitric oxide (NO) is a radical compound formed when a single nitrogen is covalently bonded with a single oxygen (·N = O). Nitric oxide is produced endogenously from the catabolism of L-arginine by a nitric oxide synthase (NOS).

The Janus Nature of Nitric Oxide: Immunotoxicant and Immunomodulator

As for most, if not all, endogenous and exogenous compounds that have differential effects on the immune or any organ system, their dose and time of exposure affect whether the outcome is positive (stimulatory) or negative (inhibitory). Originally described by Venchikov (1960) for biological effect dependent on dose, a chemical, such as iron, can have a wide biological range of effects. Based on the exposure dose, the effects can range from being essential for existence to positive physiologically stimulatory effect, to negative physiological interferences due to affects on the wrong cell type or cellular compartment, to positive pharmacological influences, and, finally, to negative toxicological effects. Oxygen, itself, as well as NO, possess this dynamic range in potential biological effects. Some of the immunobiology/pathology of NO can be direct effects on particular types of immune cells, as later described, and some can be indirect effects on the immune system, e.g., through influences on cardiovascular functions (Moncada et al. 1991), which are depicted by the synonym for NO, endothelium-derived relaxing factor, that can alter cell trafficking and distribution of soluble immunoregulatory factors. The dynamic range of NO activity is related to its "ubiquitous, highly diffusible, and promiscuously reactive" nature (Hollenberg and Cinel 2009). The ubiquitous nature of NO is due to its production by different cell types throughout the body. The monocytic lineage of the immune system, including macrophages, microglia, monocyte-derived dendritic cells, and monocytic myeloid-derived suppressor cells, has an

inducible enzyme (inducible nitric oxide synthase, iNOS or NOS2) that converts L-arginine to NO and citrulline, whereas the other isoforms of NOS (endothelial (e)NOS or NOS3 and neuronal (n)NOS or NOS1) can constitutively produce NO. In addition to the dose effects of NO on immunity, NO and its more powerful oxidant peroxynitrite ($ONOO^-$), which forms rapidly and spontaneously when NO interacts with superoxide anion (O_2^-), may differentially affect immunity dependent on whether the exposure occurs before, with, or after other immunomodulatory exposures. Although most studies of infectious diseases and tumor immunology discuss NO with regard to its killing (toxic) potential on bacteria, viral-infected cells, or cancer cells, NO is involved in several physiological processes (Pacher et al. 2007) including tissue repair processes (Witte and Barbul 2002; Schwentker and Billiar 2003) and angiogenesis (Ziche and Morbidelli 2000).

NO involvement in the immune response to pathogens and cancers was first indicated when the levels of nitrite and nitrate (NO metabolites) were shown to increase (Tannenbaum 1987; Hibbs et al. 1987). Once macrophages are activated by bacterial products such as lipopolysaccharide (LPS), other pattern-recognition receptor (PRP) stimulators, or the interferon-gamma (IFN-γ), iNOS can generate variant doses of NO (Espey et al. 2000) dependent on additional cofactors such as tetrahydrobiopterin, calmodulin, and flavin nucleotides. The ability to produce NO is also dependent on the availability of its substrate L-arginine. NO itself influences its own activity and production, respectively, by reacting with superoxide to form the more active peroxynitrite and by generating N-hydroxyarginine, which both lowers the availability of arginine (the NOS substrate for NO production) and inhibits the activity of arginase (Boucher et al. 1994), the enzyme that eliminates arginine. For infection controls, NO often can regulate or collaborate with the production of reactive oxygen species (ROS), including O_2^- and hydrogen peroxide (H_2O_2) to regulate the level of oxidative stress. The metabolic products generated by the convoluted pathways of NO and ROS interactions include a wide range of molecules with diverse functions, such as polyamines, glutathione (GSH), leukotrienes, and prostaglandins; these molecules each have their own individual positive or negative effects on immunity. The combination of NO, $ONOO^-$, and ROS maintains cytotoxic activity and immune regulation. The interplay of NO and ROS at low levels initially aids immune cell activation. They then participate in the proinflammatory response, which is needed to kill pathogens, but then they eventually must assist termination of inflammation by inducing anti-inflammatory signals. Otherwise, excessive inflammation will cause tissue damage, as apparent in tuberculoid leprosy. Discussion of ROS and antioxidant activity is in a separate section entitled "► Antioxidants."

Inducible (i) NOS

Although there are differences in the transcriptional activation between species, there are some common transcription factors including nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT)-1α, and interferon regulatory factor-1 (Pautz et al. 2010). The transcription factor NF-κB is intimately involved in the biphasic nature of the early low-level NO/ROS turn-on effects and the later higher-level turnoff of proinflammatory cytokine production. The low-level oxidative processes activate NF-κB by enhancing its dissociation from IκB, but excessive oxidative levels prevent NF-κB from binding to its promoter site and activating transcription. IL-1β and TNF-α influence NF-κB activation, whereas STAT-1 is activated by interferon-gamma (IFN-γ). NO, itself, is able to regulate iNOS expression; NO mediates superinduction and inhibition of iNOS expression (Connelly et al. 2001; Pérez-Sala et al. 2001). This dual activity, which also relates to its influence on NF-κB expression (Connelly et al. 2001), probably relates to its interactions with ROS constituents, which affect the signaling pathways being modified. Although both rodents and humans can express iNOS in cell types other than macrophages, such as epithelial cells, astrocytes, and endothelial cells, human

hepatocytes express iNOS in the absence of an infection and human neurons express iNOS with certain neurodegenerative conditions, such as Alzheimer's disease. The differences in iNOS expression relate to differences in the transcription factors and promoter region of iNOS (Weinberg 1998). Unlike eNOS and nNOS, the activity of iNOS is independent of the intracellular Ca^{2+} concentration, which is due to the fact that iNOS exists in tight association with the calcium-binding calmodulin protein. All three NOS isoforms are homodimeric hemo-flavoproteins; their N-terminal half contains the iron protoporphyrin IX (heme). This heme domain or the oxygenase domain of NOS is the site that binds the tetrahydrobiopterin cofactor and the substrate arginine. The C-terminal half of NOS is the flavin-binding domain (or reductase domain), and it contains Flavin Adenine Dinucleotide (FAD)-, Flavin Mononucleotide (FMN)-, and Nicotinamide Adenine Dinucleotide Phosphate or Glutamine Synthase (NADPH)-binding sites.

Molecular Modes of Action

Once NO is generated, it can modify many different cellular processes by S-nitrosylation, which alters the structure and function of proteins by adding NO to the free thiol of a cysteine forming a S-nitrosothiol. The thiols of other molecules, such as coenzyme A or GSH, also can be modified. Proteins critical to cell survival and proliferation can, thus, be altered, especially those of proteins that require iron for their activity, such as ribonucleotide reductase and aconitase. NO also affects the activity of soluble guanylate cyclase, Adenosine Diphosphate (ADP) ribosylation of proteins, and iron regulatory factor activation.

Different Types of Immune Cells Produce NO and Are Regulated by NO

Macrophages are the cells usually considered the main immune cell producing NO, and neutrophils are the main cell type producing ROS. However, NO from endothelial and epithelial cells and even neurons can affect immune responses; additionally, there are multiple subtypes of macrophages and cells of the monocytic lineage, and only some of them are induced to express iNOS. Classically activated (or M1 macrophages) as well as a macrophage subset that has been referred to as type II macrophages can express iNOS and produce NO (Edwards et al. 2006). Mouse M2 macrophages do not express iNOS; in fact, they express arginase, which, as stated earlier, interferes with the availability of arginine, and thus, M2 macrophages inhibit production of NO. Like iNOS, arginase is an inducible enzyme, and it has two isoforms: arginase-1 (cytosolic) and arginase-2 (mitochondria associated). M1 and M2 cells are well defined in mice, but in humans, $CD16^{-}$ and $CD16^{+}$ monocyte/macrophage subsets are functionally less well defined. Human $CD16^{+}$ macrophages are more inflammatory-mediating, but they express both iNOS and arginase. Mouse M1 macrophages release IL-12, which preferentially enhances development of Th1 cells, and Th1 cells produce IFN-γ, which in turn induces M1 macrophages to express iNOS and produce NO. On the other hand, Th2 cells release IL-4, which induces the M2 macrophage phenotype, and M2 macrophages produce IL-10 and TGF-β, which inhibit cell-mediated immune activities of Th1 and M1 macrophages. The biochemical complexities of NO and ROS interactions at times seem paradoxical in that both act together to kill pathogens via their oxidative capacity; however, O_2^{-} production declines when NOS activity and NO levels are high and NO levels inhibit oxidative mechanisms associated with ROS. ROS and peroxynitrite decrease the major antioxidant in cells, glutathione (GSH), and when GSH levels are low, M1 macrophages are inactive and M2 macrophages are active (Murata et al. 2002). With the decline in GSH, the antioxidant response element (ARE) pathway is activated, which leads to the induction of nuclear factor erythroid-2 (NRF2), which affects γ-glutamylcysteine ligase, the rate-limiting enzyme for GSH biosynthesis, and glutathione reductase.

The M1 macrophages drive the inflammatory response with their release of inflammatory cytokines (IL-1β, TNF-α, and IL-6) as well as production of NO and ROS and proteases and chemokines. However, the expression of the inflammatory cytokines is not equally affected by the level of the NO, in that TNF-α positivity correlates with NO but higher levels of NO inhibit IL-1β. The release of IL-12 from the M1 macrophages also promotes Th1 development (M1 macrophages are antigen-presenting cells (APC) for preferential development of Th1 cells), and Th1 release of IFN-γ will further enhance M1 activities. As noted, if inflammation continues much beyond the elimination of an infection, there will be further tissue damage and inhibition of tissue repair; thus, there is the need for regulation. The NO and ROS interactions affect the timing controls needed to allow an adequate amount of inflammation but not an excess; the necessary regulatory controls are mediated, in part, by influences of NO and ROS on the balance between the M1-Th1 and M2-Th2 pathways. Additionally, NO affects development of a separate subpopulation of regulatory T cells, IL-10 releasing Treg cells (Niedbala et al. 2007). However, NO also interferes with FoxP3 expression, and thus Treg development during the induction of experimental autoimmune encephalomyelitis (Brahmachari and Pahan 2010), which may relate to the differential regulatory influences of timing and dose of NO exposure. The anti-inflammatory cytokines from Th2 cells and Treg cells are IL-4, IL-13, IL-10, and TGF-β. As the activation of iNOS decreases, there is an increase in the level of the prostaglandin PGE2, which enhances the cAMP level, and cAMP aids preferential activation of Th2 cells. Interestingly, high NO levels lower the IL-1β level, and IL-1β has been suggested to favor Th2 development. The NO/ROS level also leads to activation of ARE signaling with expression of NRF2, which besides enhancing antioxidant levels enhances expression of inducible heme oxygenase (HO-1), a microsomal enzyme that cleaves heme-containing enzymes, including iNOS, and generates products that can inhibit TNF-α-mediated cell death.

The T cell subsets associated with NO induction are not restricted to the Th1 and Th2 cells. The $CD4^+$ Th subsets include Th9, Th17, and Th22 cells. Similar to Th1 cells, Th17 and Th22 have been suggested to induce iNOS expression via IL-17 and IL-22, respectively (Mühl et al. 2011). NO and ROS also may interfere with the production of IL-12 and IL-27, which promote Th1 cells, and IL-23, which promotes Th17 cells, since these cytokines are disulfide cross-linked heterodimers and loss of the free thiols of the monomers would block their formation. NO also can be immunosuppressive by enhancing the activity of other cells of the myeloid monocytic lineage. The myeloid-derived suppressor cell ($CD14^+CD15^-$ MHC class II^{low}) can produce NO, and like M2 macrophages, it releases IL-10 and TGF-β, but unlike M2 macrophages, it is not an APC.

NO and Disorders

As might be expected due to the ubiquitous nature of NO and its diversity of influences on multiple cell types, NO is involved in both exacerbation of pathologies as well as the suppression of immune-mediated pathologies and tissue repair as described earlier. Many reports have described the influence of NO in inflammatory bowel disorder, and both endogenous cells as well as the microbiome can affect the levels of NO. Mesenchymal stem cells (MSC) reduce inflammation after spinal cord injury and promote functional recovery by shifting the presence of M1 to M2 macrophages (Nakajima et al. 2012), and proinflammatory-activated MSC can produce chemokines and iNOS leading to immunosuppression of T cell proliferation (Ren et al. 2008). The compartmentalization of NO in the cells of various organs or lack thereof relates to the resolution or mediation of several clinical conditions (Villanueva and Giulivi 2010).

Cross-References

► Antioxidants
► Autoinflammatory Diseases

▶ Prostaglandins, Leukotrienes, and Related Compounds
▶ Resolution of Inflammation
▶ Systemic Lupus Erythematosus, Pathogenesis

References

Boucher JL, Custot J, Vadon S, Delaforge M, Lepoivre M, Tenu JP, Yapo A, Mansuy D. N omega-hydroxyl-L-arginine, an intermediate in the L-arginine to nitric oxide pathway, is a strong inhibitor of liver and macrophage arginase. Biochem Biophys Res Commun. 1994;203:1614–21.

Brahmachari S, Pahan K. Myelin basic protein priming reduces the expression of Foxp3 in T cells via nitric oxide. J Immunol. 2010;184:1799–809.

Connelly L, Palacios-Callender M, Ameixa C, Moncada S, Hobbs AJ. Biphasic regulation of NF-kappa B activity underlies the pro- and anti-inflammatory actions of nitric oxide. J Immunol. 2001;166:3873–81.

Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. J Leukoc Biol. 2006;80(6):1298–307.

Espey MG, Miranda KM, Pluta RM, Wink DA. Nitrosative capacity of macrophages is dependent on nitric-oxide synthase induction signals. J Biol Chem. 2000;275:11341–7.

Hibbs Jr JB, Taintor RR, Vavrin Z. Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Science. 1987;235:473–6.

Hollenberg SM, Cinel I. Bench-to-bedside review: nitric oxide in critical illness – update 2008. Crit Care. 2009;13(4):218.

Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev. 1991;43:109–42.

Mühl H, Malte Bachmann M, Pfeilschifter J. Inducible NO synthase and antibacterial host defence in times of Th17/Th22/T22 immunity. Cell Microbiol. 2011;13(3):340–8.

Murata Y, Shimamura T, Hamuro J. The population of T (h)1/T(h)2 balance is dependent on the intracellular thiol redox status of macrophages due to the distinctive cytokine production. Int Immunol. 2002;14:201–2.

Nakajima H, Uchida K, Rodriguez Guerrero A, Watanabe S, Sugita D, Takeura N, Yoshida A, Long G, Wright K, Johnson E, Baba H. Transplantation of mesenchymal stem cells promotes the alternative pathway of macrophage activation and functional recovery after spinal cord injury. J Neurotrauma. 2012;29(8):1614–25.

Niedbala W, Cai B, Liu H, Pitman N, Chang L, Liew FY. Nitric oxide induces $CD4^+$ $CD25^+$ FoxP3 regulatory T cells from $CD4^+$ CD25 T cells via p53, IL2 and OX40. Proc Natl Acad Sci USA. 2007;104:15478–83.

Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007;87(1):315–424.

Pautz A, Art J, Hahn S, Nowag S, Voss C, Kleinert H. Regulation of the expression of inducible nitric oxide synthase. Nitric Oxide. 2010;23(2):75–93.

Pérez-Sala D, Cernuda-Morollón E, Díaz-Cazorla M, Rodríguez-Pascual F, Lamas S. Posttranscriptional regulation of human iNOS by the NO/cGMP pathway. Am J Physiol Renal Physiol. 2001;280:F466–73.

Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008;2:141–50.

Schwentker A, Billiar TR. Nitric oxide and wound repair. Surg Clin North Am. 2003;83:521–30.

Tannenbaum SR. Endogenous formation of N-nitroso compounds: a current perspective. IARC Sci Publ. 1987;84:292–6.

Venchikov AI. Physiologically active quantities of a microelement. Voprosy Pitaniya. 1960;19:3–11.

Villanueva C, Giulivi C. Subcellular and cellular locations of nitric-oxide synthase isoforms as determinants of health and disease. Free Radic Biol Med. 2010;49:307–16.

Weinberg JB. Nitric oxide production and nitric oxide synthase type 2 expression by human mononuclear phagocytes: a review. Mol Med. 1998;4:557–91.

Witte MB, Barbul A. Role of nitric oxide in wound repair. Am J Surg. 2002;183:406–12.

Ziche M, Morbidelli L. Nitric oxide and angiogenesis. J Neurooncol. 2000;50:139–48.

NK Cell Activation

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Definition

Natural killer (NK) cells are a third subpopulation of lymphocytes. They are part of the innate immune system, but also contribute to the adaptive immune response. In 1975, NK cells were identified by their ability to lyse allogeneic tumor cells in mice without prior sensitization. The term “natural cytotoxicity” was introduced to describe this activity, and subsequently the

cells mediating this effect were termed natural killer cells. NK cells and T cells both derive from the common lymphocyte progenitor, but in contrast to T cells, NK cells develop in the bone marrow. They are not dependent on a functional thymus but need cytokines like interleukin (IL-) 2 and IL-15 for differentiation. Unlike T and B cells, NK cells express germ-line-encoded surface receptors and do not undergo somatic recombination (Sun and Lanier 2011).

NK Subsets

NK cells constitute about 5–15 % of peripheral blood lymphocytes. Further, they circulate through several lymphoid and non-lymphoid tissues like liver, spleen, tonsils, or lung. According to their function and phenotype, distinct subsets of human NK cells were defined: $CD16^{+}$ $CD56^{dim}$ NK cells are mainly found in peripheral blood and are assumed to be the mature, cytotoxic NK subtype. In contrast, $CD16^{-}$ $CD56^{bright}$ NK cells are preferentially located in secondary lymphoid organs and are thought to be the main producers of cytokines (Sun and Lanier 2011). However, both NK subsets can actually fulfill both functions. Uterine NK cells (Manaster and Mandelboim 2010) constitute another specialized subset. They secrete a variety of cytokines, growth factors, and angiogenic factors that might promote implantation and vascularization during early pregnancy. Innate lymphocytes sharing several features with NK cells are found in the intestinal mucosa. These cells do not exhibit cytotoxic activity but instead secrete IL-22 and are involved in mucosal homeostasis (Sanos et al. 2011).

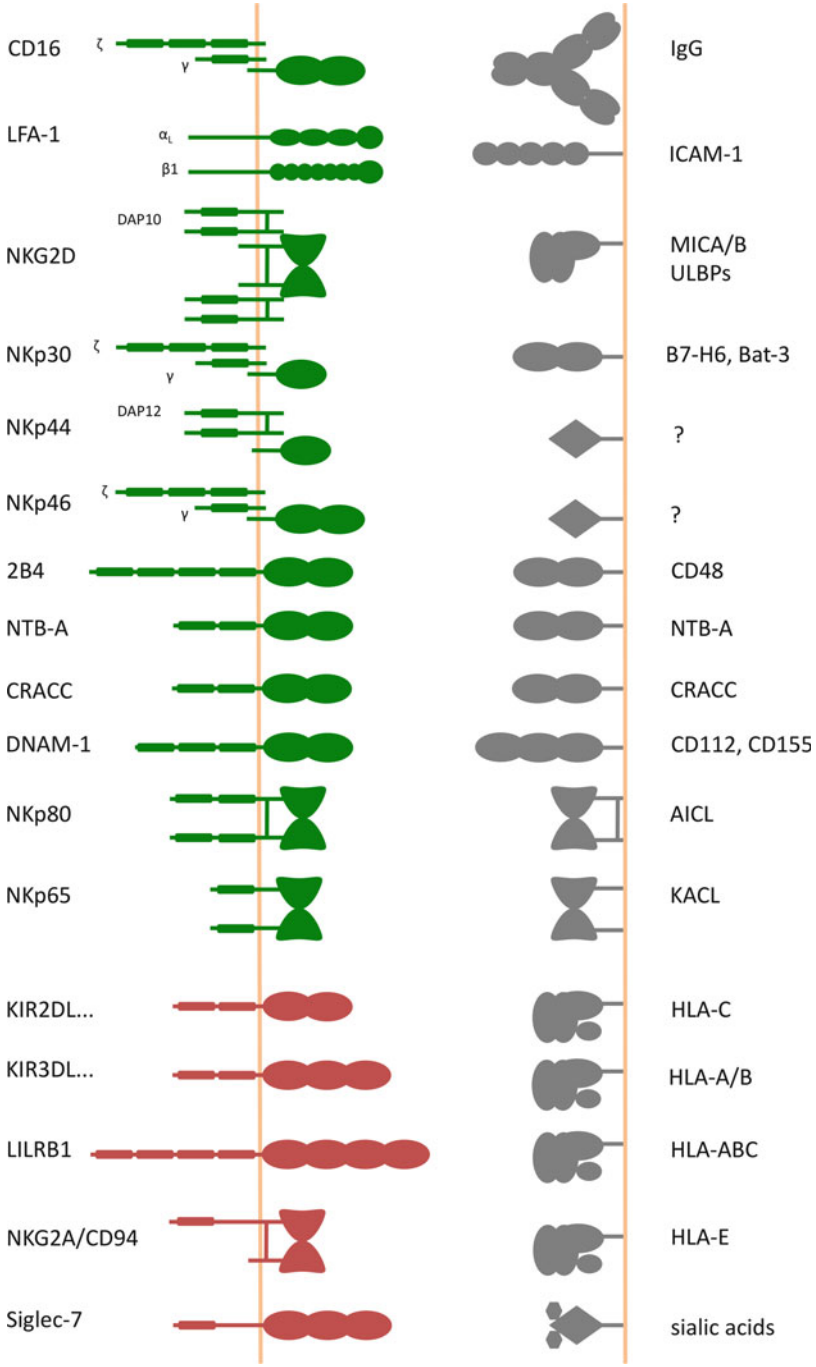
NK Cell Functions

NK cells provide a first line of defense against parasites, viruses, and cancer (Chavez-Galan et al. 2009; Lodoen and Lanier 2006; Vesely et al. 2011). One feature of NK cells is their cytotoxic activity against infected or transformed cells. NK cells carry large numbers of preformed cytotoxic granules. Upon binding to the target and activation of the NK cell, granule content is released towards the target and induces target cell apoptosis. The mechanisms of synapse formation and target cell killing share some properties with cytotoxic T lymphocytes.

After stimulation by direct target cell contact or through cytokines like IL-12 or IL-18, NK cells produce several mainly proinflammatory cytokines. NK cells play a crucial role in the direct control of viral replication and contribute to the communication between innate and adaptive immune system. Especially IFN-γ and TNF-α are essential for tuning the T cell-mediated response against intracellular pathogens and tumors. NK cell helper function is vital for the crosstalk with dendritic cells, either by direct contact or by cytokine release (Moretta et al. 2008).

Inhibitory Receptors: Self-Tolerance and Education

NK cell activity is regulated by various activating and inhibitory surface receptors (Fig. 1). Since several ligands for activating NK cell receptors are also expressed on normal, healthy cells, NK cell activity has to be inhibited by healthy tissue to avoid auto-reactivity. NK cells express inhibitory killer cell immunoglobulin-like receptors (KIR) and the Ig-like LILRB1 that recognize MHC class Ia and Ib molecules, and the NKG2A/CD94 heterodimer, which binds HLA-E. KIR contain two (KIR2D) or three (KIR3D) Ig-like extracellular domains and immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their long intracellular part. Different KIR specifically recognize distinct human leukocyte antigen (HLA)-A, (HLA)-B, or (HLA)-C allotypes, but in contrast to the TCR, binding is, for the most part, not peptide specific. There are also activating KIR with a short intracellular tail, which transduces signals via immunoreceptor tyrosine-based activation motif (ITAM) containing adapters. However, the role of activating KIR variants for NK cell function is still not entirely clear.



NK Cell Activation, Fig. 1 Activating and inhibitory NK cell receptors and their ligands. The upper part shows a schematic representation of several important activating NK cell receptors. Some of these receptors couple to signaling partner chains such as DAP10, DAP12, CD3ζ, or FcεRIγ; others have signaling motifs in their cytoplasmatic domain. Cellular ligands for some of the receptors are still unknown. The lower part shows a summary of inhibitory receptors. KIR are specific for certain HLA alleles; LILRB1 binds a wide range of HLA alleles. Siglec-7 recognizes sialic acid modifications

During viral infection or tumorigenesis MHC class I expression is often downregulated or even lost from the cell surface to evade T cell-mediated responses. However, NK cells can be activated by these MHC I low target cells. This feature led to the development of the so-called missing self hypothesis. Yet, target cells that do not express any functional NK cell ligands for activating (or inhibitory) receptors are not recognized at all. The presence of stimulating ligands is

indispensable to fully activate NK cells. Some of these ligands are constitutively expressed on all cells; some are upregulated upon virus infection, tumor transformation, or cellular stress. Increased expression levels of such ligands can overcome dominant inhibitory signals and finally lead to induction of cytotoxicity even in the presence of self-MHC class I molecules. This concept has been termed "induced self" (Elliott and Yokoyama 2011).

However, NK cells are not overreactive in humans or mice deficient in expression of MHC class I. Instead, NK cells which developed in this MHC class I-deficient environment are hyporesponsive to MHC class I-deficient targets. Therefore, NK cells can sense which host MHC class I alleles are expressed in their environment and if there are compatible inhibitory KIR on the particular NK cell. NK cells that do not express at least one inhibitory receptor that fits to the MHC class I molecules expressed as self are functionally incompetent. The flexibility of this system is underscored by the finding that hyporesponsive mouse NK cells from an MHC-deficient donor can regain their reactivity after transfer into an MHC-sufficient recipient. The mechanisms behind this process are still not fully understood. Several models have been proposed which are summarized under the terms "MHC class I-dependent education" or "licensing" (Elliott and Yokoyama 2011). Recent data have suggested that this mechanism is not a static "yes-no" system but that the potency of an NK cell response is dependent on the strength of the "licensing" input. An NK cell that expresses multiple receptors for inhibitory self-ligands receives strong licensing signals. This NK cell would generate stronger activating responses than NK cells with less or weaker inhibitory contacts. Yet, NK cells that lack inhibitory receptors, and are therefore per definition "unlicensed," are apparently not completely unresponsive: Mouse NK cells without any inhibitory receptors were recently identified as the major producers of cytokines in response to infection with MCMV (Orr and Lanier 2011).

Activating Receptors

The panel of activating NK cell receptors is very heterogeneous. They cover several receptor families that recognize a large variety of different ligands and use different signaling pathways to transduce activating signals (Moretta et al. 2001). CD16 (FcγRIIIA) has a distinctive role among the receptors, as it does not bind to cellular ligands but triggers antibody-dependent cellular cytotoxicity (ADCC) upon binding to the Fc part of antibody complexes or antibodies bound to target cells. CD16 is the only NK receptor that can induce cytotoxicity without co-stimulation by other receptors in resting NK cells. Further, NK cells express members of the Ig-like SLAM-related-receptor family (SRR), including 2B4, NTB-A, and CRACC (Claus et al. 2008). While NTB-A and CRACC are homophilic receptors, 2B4 binds a distinct ligand, the GPI-anchored Ig-like protein CD48, that is expressed on all hematopoietic cells. SRR are not confined to NK cells but are also expressed on B and T cells, allowing for crosstalk between these cell types. The homodimer NKG2D belongs to the C-type lectin family and recognizes a variety of stress-induced ligands such as MICA and MICB and proteins of the ULBP family. NKp65 binds to KACL on keratinocytes. NKp80 is another member of this family that recognizes AICL, which is upregulated on myeloid cells upon TLR stimulation. DNAM-1 binds PVR and nectin-1, both expressed on tumor cells. Finally, the Ig-like natural cytotoxicity receptors (NCRs) NKp30, NKp44, and NKp46 are important receptors that induce strong activation signals. NKp30 and NKp46 are expressed on all NK cells, while NKp44 expression is induced upon NK cell activation. Although the importance of NCRs for tumor rejection has been demonstrated, only cellular ligands for NKp30 have been identified so far. The Ig-like protein B7-H6 is a tumor-specific surface marker, whereas the nuclear chaperone BAT-3 can be released from tumor cells and dendritic cells in exosomes and vesicles. It is known that NCR ligands are expressed on many different tumor cells and that these receptors can bind to heparan sulfate moieties and recognize different

viral hemagglutinins on infected cells, and they may also interact with bacterial proteins (Chaushu et al. 2012). NK cell activity is further modulated by proinflammatory cytokines, ▶ chemokines, and other soluble agents (Moretta et al. 2008).

First Contact: Adhesion and Initial Signaling

In the following section we will describe the signaling events during NK cell activation upon encounter of a target cell. We will describe these events in chronological order, starting from early adhesion events to the lysis of the target cell.

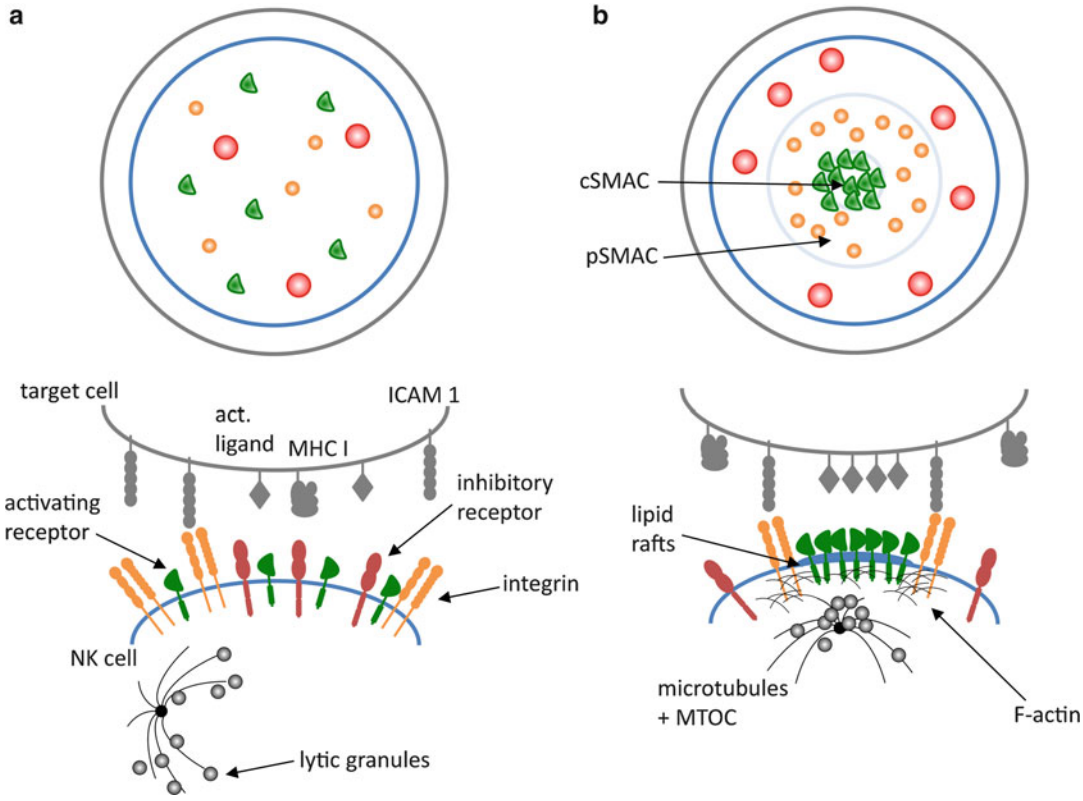
Upon first contact between an NK cell and a potential target cell, selectins (L-selectin) and integrins (LFA-1, MAC-1) (▶ Cell Adhesion Molecules) can loosely bind to the target and thus promote initial adhesion and signaling. The integrin LFA-1 (CD11a;CD18, αL;β1) (Hogg et al. 2011) recognizes the adhesion molecules ICAM-1, ICAM-2, and ICAM-3, which are expressed on endothelial cells and are upregulated during inflammation. In contrast to T cells, isolated triggering of LFA-1 on NK cells is sufficient to induce early activation signals. These drive polarization and directed recruitment of activating NK cell receptors towards the contact site and facilitate scanning for the presence of additional activating ligands. Simultaneous engagement of inhibitory receptors by MHC class I ligands interferes with NK cell activation already at this early signaling stage.

The adhesion strength of LFA-1 is determined by the affinity and avidity for ICAM (Hogg et al. 2011). The cytoplasmic domain of β1 integrin associates with adapter proteins like talin that link the integrin to the actin cytoskeleton and to downstream signals that arrive from other activating receptors. This "inside-out" signaling induces conformational changes in the intracellular part of the LFA-1 heterodimer. These are passed through the transmembrane segments and result in the formation of a high-affinity conformation of LFA-1, which strengthens the initial interaction between effector and target cell. Engaged LFA-1 in turn is able to transduce signals into the cell via outside-in signaling (Hogg et al. 2011). Concerted signaling of the different activating receptors promotes reorganization of the actin cytoskeleton, actin-dependent clustering of membrane microdomains, and recruitment of additional receptors and adapters into these signaling platforms, finally leading to the formation of a highly ordered and tight interface between effector and target, called immunological synapse (IS) (Fig. 2).

The IS is organized in discrete concentric regions that are defined by the accumulation of distinct proteins into supramolecular activation clusters (SMACs): the central cSMAC is constituted by activating receptors and their corresponding adapters and signaling molecules. This area is enclosed by the peripheral pSMAC, consisting of an F-actin ring and adhesion molecules such as LFA-1. The surrounding zone where inhibitory receptors or CD45, which are excluded from the IS, are found is sometimes referred to as distal dSMAC (Orange 2008). It has been shown that signaling microclusters do also form outside the IS. But for directed NK cell cytotoxicity, the functional IS is crucial as it ensures close contact to the target cell, and it provides the platform for signal amplification by concentrating receptors, co-stimulators, and cytoplasmic signaling molecules. In the mature IS, activating and adhesion signaling is further amplified until the activation threshold is reached and the cytolytic machinery is turned on.

Signaling of Inhibitory Receptors

A common signaling motif for inhibitory mouse Ly49, human KIR, and the conserved NKG2/CD94 receptors is the ITIM in the cytoplasmatic tail of these receptors (Watzl and Long 2010; Watzl and Urlaub 2011). Upon ligand binding, the ITIMs are tyrosine phosphorylated and can recruit the protein tyrosine phosphatases SHP-1 and SHP-2 and the SH2-domain-containing inositol phosphatase (SHIP). This binding to the receptors, and probably also phosphorylation,

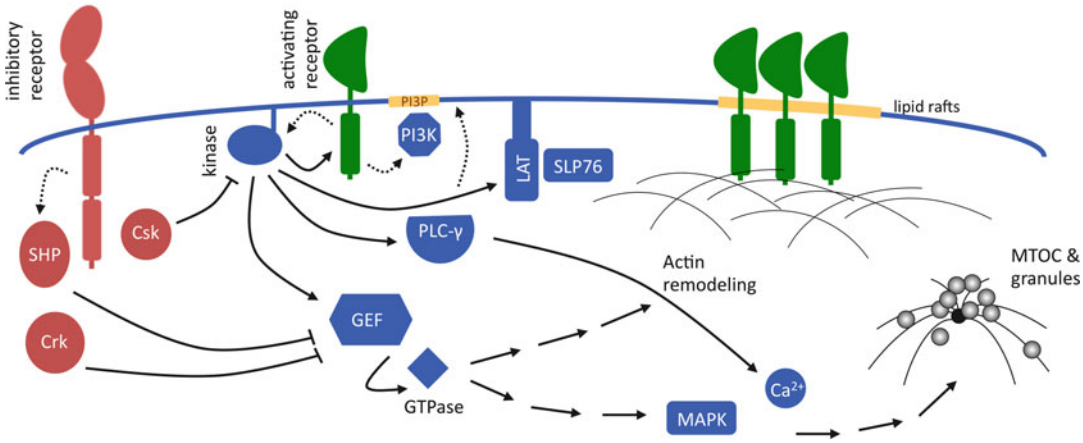


NK Cell Activation, Fig. 2 Immunological synapse. (**a**) First contact between NK cell and target cell involves adhesion molecules like LFA-1 and activating and inhibitory receptors, which are diffusely distributed on the cell surface. (**b**) Dominant activating signals lead to synapse formation, F-actin remodeling, recruitment of activating receptors into membrane microdomains, and signal amplification. Lytic granules and MTOC polarize towards the target cell

increases the activity of the phosphatases, which is considered a major mechanism necessary for NK cell inhibition. Other signaling events that contribute to ITIM-mediated inhibition include the phosphorylation of the small adapter Crk. This leads to an active disassembly of activating signaling complexes containing c-Cbl and the GTP-exchange-factor (GEF) C3G. Additionally, the C-terminal Src kinase (Csk) phosphorylates the inhibitory tyrosine of Src-family kinases, which stabilizes their inactive conformation.

Signaling of Activating Receptors

The multitude of activating NK cell receptors uses different signaling motifs and partner chains (Fig. 3). Resulting from this diversity, receptor proximal signaling events are also very variable and can trigger different signaling pathways (Watzl and Long 2010; Watzl and Urlaub 2011). Receptors that are associated to ITAM-based signaling partners induce signals similar to B or T cell activation. Engagement of the receptors CD16, NKp30, and NKp46 that are coupled to the CD3ζ chain or NKp44, which signals via DAP12, results in Src-family-kinase-dependent phosphorylation of the associated molecules' ITAMs. Once these motifs are phosphorylated, they can recruit the kinases ZAP70 and Syk, which in turn activate adapter proteins like ▶ LAT, LAT2, or SLP76 by phosphorylation. Emanating from the adapter proteins, signaling complexes are assembled, leading to the activation of further pathways. GEFs like Vav are important connections to cytoskeleton



NK Cell Activation, Fig. 3 Signaling pathways of NK cell receptors. Activating receptors initiate signals via various pathways. Important mediators are GTPases, the MAPK cascade, and Ca^{2+} signals, leading to cytoskeleton remodeling, polarization of the lytic machinery, and degranulation. Inhibitory receptors can intercept these signals at early steps

remodeling. ITAM signals mainly activate the Vav family members 2 and 3.

Other NK cell receptors or their associated signaling molecules contain tyrosine-based motifs different from ITAMs. The NKG2D receptor can also signal via DAP12 in mice, but in humans exclusively couples to DAP10, which contains a YINM motif. Similar to ITAMs, this motif can be phosphorylated by Src-family kinases and recruit phosphatidyl-inositol-3-OH kinase (▶ PI3K) or the adapter Grb2, which leads to the recruitment of Vav1.

SLAM-family receptors do not couple to signaling partner chains, but possess their own typical signaling motif, called immunoreceptor tyrosine-based switch motif (ITSM) (Claus et al. 2008). This motif can also be phosphorylated by Src-family kinases and recruits small SH2 domain-containing adapter molecules SAP, EAT-2, and in mice additionally ERT. SAP can bind to the Src-family kinase Fyn, which is probably responsible for activating signals transmitted by the SLAM-family receptors 2B4 and NTB-A that include phospholipase C (PLC)-γ1 and Vav1. The receptor CRACC is independent of SAP but interacts with EAT-2. It is still unclear how EAT-2 can contribute to NK cell activation. The ITSM motif has another special property: the ability to bind phosphatases (SHP-1 and SHP-2, ▶ SH2 Domain-containing Inositol Phosphatase-1 (SHIP)) instead of the small adapter molecules. This interaction explains how these receptors can transmit inhibitory signals, which can be observed under certain conditions. In X-linked lymphoproliferative syndrome (XLP) patients, the lack of functional SAP can cause an inhibitory function of 2B4. In NK cell precursors, which do not yet express MHC I-specific inhibitory receptors, the SAP level is very low. Inhibitory 2B4 signals could maintain the tolerance of these cells.

Other receptors such as DNAM-1, NKp80, or NKp65 have different tyrosine-based signaling motifs in their cytoplasmic tails, which have not yet been investigated in detail. In addition to their important role in adhesion to the prospective target cell, integrins have also the ability to transmit an outside-in signal. These signals also lead to the activation of Vav1 and play a major role in polarization of the lytic machinery towards the target (Watzl and Long 2010).

Downstream Activation

Despite the multitude of activating NK cell receptors and their various signaling motifs,

there are many common downstream signaling pathways. Src-family kinases are involved in the induction of most of these signaling events by phosphorylating the various tyrosine-based signaling motifs. These kinases therefore account for the recruitment of subsequent signaling mediators, but often they are also necessary to activate these mediators by phosphorylation. Only ITAM-based receptors additionally need the kinases Syk and ZAP70 to transmit the signal. Phosphatidyl-inositol-3-OH kinase (PI3K) can be activated either by direct binding to receptor complexes or by secondary events. By increasing the amount of phosphatidyl-inositol-3,4,5-triphosphate (PI3P), active PI3K enables the recruitment of Tec family kinases, PLC-γ1 or PLC-γ2, Akt, and Vav1, to the stimulated receptor. The induction of Ca^{2+} flux via PLC-γ is one of the essential events that are triggered by many receptors, as it is necessary for the exocytosis of lytic granules. Vav1 contributes to the activation of Rho-family ► GTPases like Rac1 and Cdc42 that are important regulators of actin reorganization. The MAP kinase ERK is activated in pathways following PI3K-Akt and Vav1-Rac1 and plays a key role in initializing granule polarization and release (Orange 2008; Watzl and Urlaub 2011).

The reorganization of the actin cytoskeleton is central for the activation of NK cells. The actin-dependent clustering of activating receptors at the IS creates an amplification of activating signals (Orange 2008). The process of activating receptor clustering is paralleled by an accumulation of cholesterol-enriched membrane microdomains at the IS. The environment of these microdomains seems to be crucial for the phosphorylation and function of activating receptors.

In a physiological setting, NK cells receive a multitude of signals via a combination of activating receptors. Some of these signals are able to synergize in the activation of human NK cells, while other combinations merely show an additive effect. Uncovering the complex signal integration of this crosstalk still is in a very early stage (Watzl and Urlaub 2011).

Integration of Activating and Inhibitory Signals

NK cell activation can be blocked at a very early step if inhibitory receptors are triggered simultaneously (Watzl and Long 2010; Watzl and Urlaub 2011). Already the initial adhesion to a prospective target is affected by inhibitory signals through the regulation of integrin affinity. The induction of the high-affinity conformation of LFA-1 by different activating NK cell receptors via inside-out signaling is susceptible to inhibitory signals. The recruitment of activating receptors such as 2B4 and NKG2D to membrane microdomains can also be blocked by inhibitory signals thereby depriving activating receptors of the environment required for phosphorylation. These results show that inhibitory receptors can interfere with the earliest stages of NK cell activation. As a result, later events including the phosphorylation of various signaling molecules, Ca^{2+} flux, the polarization of lytic granules, and ultimately degranulation cannot be initiated.

These effects of inhibitory signals are closely linked to their influence on actin dynamics. Triggering of inhibitory receptors prevents the accumulation of actin at the IS which is necessary for the clustering of activating receptors. In contrast to activating receptors, inhibitory interactions can occur independently from actin and membrane microdomain rearrangement. The inhibitory event is spatially restricted to individual contact sites and does not affect the entire cell. An NK cell that is simultaneously attached to a resistant and a susceptible target cell can still recognize and attack the susceptible target.

The question of how inhibitory receptors effectively block the signals mediated by many different activating receptors and combinations of these receptors is a major question when it comes to understanding NK cell function. SHIP is recruited to various inhibitory receptors and reduces the PI3P level, thereby affecting the recruitment of activation mediators. Src-family kinases are playing a central role in signal initiation of activating and inhibitory receptors, and

their activity is depending on phosphorylation states. The C-terminal phosphorylation can keep the kinase in a closed, inactive conformation. This site is phosphorylated by Csk and can be dephosphorylated by the phosphatase CD45. In contrast to T cells, this phosphatase is not essential for the activation process in NK cells. Phosphorylation of the catalytic site of Src-family kinases increases their activity, but neither for NK nor T cells, a stimulation-dependent induction of this phosphorylation could be shown. Probably the steady-state activity of these kinases is sufficient for signal initiation and therefore not subject to regulation (Watzl and Long 2010).

The GEF Vav1 is phosphorylated early after stimulation of activating receptors and has been identified as a direct target for SHP-1 when recruited by an inhibitory NK cell receptor. This may therefore represent an important integration step for activating and inhibitory signals. Phosphorylated Vav1 serves as GEF for Rho-family GTPases, which mediate many events essential for NK cell activation, like actin polymerization, recruitment of activating receptors, and IS formation. This process can create a positive feedback loop, as the early phosphorylation of Vav1 would result in actin polymerization, recruitment of activating receptors, and the proper formation of the IS. The increased number of activating receptors would further enhance Vav1 phosphorylation. By dephosphorylating Vav1 via the phosphatase SHP-1, inhibitory signals could prevent polarization of activating receptors and signaling components towards the target. A second regulatory mechanism, also targeting the polarization of the NK cell, is the phosphorylation of Crk after stimulation of inhibitory receptors. Complexes of Crk, c-Cbl, and C3G, which are also regulators of actin dynamics, are disassembled after Crk phosphorylation. These mechanisms would be consistent with the observation that blocking the polarization of the NK cell towards the target is the focus of inhibitory signals. Additionally this could explain how the cell manages to restrict the inhibitory signal to a confined region within the cell, thereby still allowing the killing of other sensitive targets (Watzl and Urlaub 2011).

These inhibitory pathways have the potential to control NK cell activation mediated by a multitude of activating receptors as they affect signaling events that are central to NK cell activation. However, it cannot be excluded that other events that act in parallel also contribute to NK cell inhibition.

Cytoskeleton: Essential for Activation and Degranulation

Reorganization of the actin cytoskeleton is essential for NK cell activation. An activating feedback loop between stimuli causing actin polarization and F-actin stabilizing the contact and recruiting signaling mediators initiates the activation process. GEFs like Vav1 and C3G are important mediators, as they transduce incoming signals to small GTPases (Watzl and Long 2010). These signals are necessary for the correct recruitment and activation of Wiskott-Aldrich syndrome protein (WASp), which is involved in the proper actin polymerization at the IS.

Once the activation threshold is reached, many downstream signaling pathways act in concert. The microtubule organizing center (MTOC) can polarize towards the IS and the lytic granules move along the microtubules in a dynein-dependent manner towards the MTOC (Orange 2008). The Cdc42-interacting protein 4 (CIP4) probably acts as an important link between WASp and the MTOC. The attachment of microtubule plus ends to the F-actin meshwork at the IS might also be involved in correct relocalization of the MTOC. PLC-γ triggers a calcium signal, including the influx of extracellular Ca^{2+} via the channel ORAI1, which is essential for degranulation, but not polarization of lytic granules. The mitogen-activated protein kinase (MAPK) pathway becomes activated, depending on the activity of small GTPases like Rac or Ras. Especially activation of the MAPKs JNK and ERK by phosphorylation is required for cytotoxicity (Orange 2008).

The actin cortex at the IS is very dense, but nevertheless it is possible for the granules to pass through this mesh. It has recently been shown that the granules can fuse with the membrane in areas with low actin density (Sanborn and Orange 2010). As the motor protein Myosin IIa is also necessary for NK cell degranulation, this passage is probably an active process.

Defects in the degranulation mechanisms result in a disease called familial hemophagocytic lymphohistiocytosis (FHL) (Filipovich 2011). Analysis of patients suffering from this syndrome identified several proteins which are essential for proper degranulation. Rab27a is required for the docking of granules to the membrane. SNARE proteins and their interacting partners are important regulators of the fusion step. MUNC13-4 and syntaxin11 are necessary for the docking and fusion process.

Important cytotoxic effectors in the lytic granules are perforin, granzymes (Chavez-Galan et al. 2009), and the saposin-like granulysin. The acidic pH and the proteoglycan matrix inside the granules keep the effectors inactive before degranulation. Perforin can insert in the target cells' membrane and form pores by polymerization. This is essential for granzyme-mediated killing of the target, although the exact mechanism is still debated. It has been speculated that granzymes simply diffuse through the perforin pores. But recent results suggest that they are taken up by endocytosis, which is probably linked to perforin-induced damage signals. Inside the target, granzymes activate apoptotic pathways through their serine protease activity. They make use of different pathways, some granzymes cleaving multiple targets, to prevent evasion by tumor cells. One of the most abundant family members, granzyme B, activates caspases but can also cause apoptosis via the mitochondrial pathway and via DNA damage (Chavez-Galan et al. 2009). A different way to kill target cells is the triggering of death receptors, which also induces apoptosis of these cells. Death receptors like ▶ Fas (CD95) and TRAIL-R are stimulated in a cell contact-dependent manner, whereas the TNFR bind the soluble cytokine TNF-α.

Outlook

NK cells are defined by their name as cytotoxic effector cells. This entry has therefore focused on this prominent and impressive activity of these cells. However, NK cells are much more than just innate killers. They combat infections and cancer and regulate adaptive immunity through a variety of mechanisms including direct cellular interactions and the secretion of cytokines and chemokines. They even have the capacity to memory-like responses. Additionally, their activity is not only regulated by target cell contact but also influenced by different cytokines. Therefore, the signaling events leading to target cell lysis as described above are just the beginning when it comes to understanding NK cell regulation.

References

Chaushu S, Wilensky A, Gur C, Shapira L, Elboim M, Halftek G, Polak D, Achdout H, Bachrach G, Mandelboim O. Direct recognition of Fusobacterium nucleatum by the NK cell natural cytotoxicity receptor NKp46 aggravates periodontal disease. PLoS Pathog. 2012;8:e1002601.

Chavez-Galan L, Arenas-Del Angel MC, Zenteno E, Chavez R, Lascurain R. Cell death mechanisms induced by cytotoxic lymphocytes. Cell Mol Immunol. 2009;6:15–25.

Claus M, Meinke S, Bhat R, Watzl C. Regulation of NK cell activity by 2B4, NTB-A and CRACC. Front Biosci. 2008;13:956–65.

Elliott JM, Yokoyama WM. Unifying concepts of MHC-dependent natural killer cell education. Trends Immunol. 2011;32:364–72.

Filipovich AH. The expanding spectrum of hemophagocytic lymphohistiocytosis. Curr Opin Allergy Clin Immunol. 2011;11:512–6.

Hogg N, Patzak I, Willenbrock F. The insider's guide to leukocyte integrin signalling and function. Nat Rev Immunol. 2011;11:416–26.

Lodoen MB, Lanier LL. Natural killer cells as an initial defense against pathogens. Curr Opin Immunol. 2006;18:391–8.

Manaster I, Mandelboim O. The unique properties of uterine NK cells. Am J Reprod Immunol. 2010;63:434–44.

Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, Biassoni R, Moretta L. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol. 2001;19:197–223.

Moretta A, Marcenaro E, Parolini S, Ferlazzo G, Moretta L. NK cells at the interface between innate and adaptive immunity. Cell Death Differ. 2008;15:226–33.
Orange JS. Formation and function of the lytic NK-cell immunological synapse. Nat Rev Immunol. 2008;8:713–25.
Orr MT, Lanier LL. Natural killer cell licensing during viral infection. Adv Exp Med Biol. 2011;780:37–44.
Sanborn KB, Orange JS. Navigating barriers: the challenge of directed secretion at the natural killer cell lytic immunological synapse. J Clin Immunol. 2010;30:358–63.
Sanos SL, Vonarbourg C, Mortha A, Diefenbach A. Control of epithelial cell function by interleukin-22-producing RORgammat + innate lymphoid cells. Immunology. 2011;132:453–65.
Sun JC, Lanier LL. NK cell development, homeostasis and function: parallels with CD8 T cells. Nat Rev Immunol. 2011;11:645–57.
Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011;29:235–71.
Watzl C, Long EO. Signal transduction during activation and inhibition of natural killer cells. Curr Protoc Immunol. 2010; Chapter 11: Unit 11 9B.
Watzl C, Urlaub D. Molecular mechanisms of natural killer cell regulation. Front Biosci. 2011;17:1418–32.

Normal Immune Function and Barrier: Defensins

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Synonyms

Antimicrobial peptides; Defensins; Natural antibiotic peptides

Definition

Defensins are cationic polypeptides of fewer than 100 amino acids that possess a characteristic β-sheet-rich fold and a framework of six disulfide-linked cysteines (Ganz 2003). They are ubiquitously present on the skin, as well as being found in bowel and lung, and have potent antimicrobial activity. As such they form an essential part of the cutaneous innate immune system.

Historical Background

Defensins were first reported in rabbit leukocytes in the 1960s (Zeya and Spitznagel 1963), but since that time have been found not only in humans, but in all mammalian species for which they have been sought, and even in plants (Carvalho Ade and Gomes 2011). In addition, in the animal kingdom, and in humans, their presence is not just limited to leukocytes but they are also secreted by epithelial cells. It is therefore believed that they occurred very early from an evolutionary standpoint (Oppenheim et al. 2003; Zhu and Gao 2013). On the basis of the number of amino acids and structural differences in disulfide bonding, defensins are classified into alpha, beta, and theta categories. Both alpha- and beta-defensins are found in humans but theta-defensins are not. In humans, alpha-defensins are found in neutrophils, some macrophages, and Paneth cells of the small intestine (Kaushansky and Williams 2010). Beta-defensins are the defensins found in skin (Bolognia et al. 2012).

Function

Beta-defensins, along with cathelicidins, a related but structurally distinct set of antimicrobial peptides, were originally thought to operate in the skin as a barrier against microbacteria passing through the stratum corneum. They have broad activity, disrupting cell membranes of both gram-positive and gram-negative bacteria (Wolff and Fitzpatrick 2008), fungi, and viral envelopes. The exact mechanism by which they do this is poorly understood and is believed to be multifaceted. One mechanism is believed to depend on their strong positive charge which allows them to bind to cell membranes of bacteria which are negative due to lipopolysaccharides creating pores in the bacterial membrane through

which cell contents can escape destroying cell homeostasis (White et al. 1995). However, it is unclear how they attack many fungi and why they are nontoxic to host cells.

However, there is strong and growing evidence that these peptides not only act as passive endogenous antibiotics but are inducible upon exposure blocking infection before it occurs as well as fulfilling additional roles unrelated to infection (Niyonsaba et al. 2006). In alpha-defensins there is binding to the CXCR4 receptor which is essential for HIV infection of the cell thereby blocking viral entry (Levinson 2006); however, beta-defensins are also induced by exposure to HIV (Niyonsaba et al. 2006). Beta-defensins are also implicated via CCR-6 signaling as chemoattractants resulting in recruitment of immature dendritic cells and memory T cells to sites of wounds or infection (Yang et al. 1999) and there is some suggestion they may be involved in wound healing, initiation of angiogenesis, and reepithelization.

Dysregulation in Disease States

In addition to the normal homeostasis and immunity within the skin, defensins have been shown to have an important role in a number of cutaneous and mucosal disease states including atopic dermatitis where defensins are significantly downregulated providing one possible explanation of why patients with atopic dermatitis are prone to bacterial infections, primarily *Staphylococcus aureus*, as well as warts, molluscum, and HSV (Rich 2013; Schauber and Gallo 2009). Defensin levels have also been shown to be altered in psoriasis and rosacea (Niyonsaba et al. 2006). In additional defensin profiles are altered in the saliva of patients with primary Sjögren's syndrome (Peluso et al. 2007) suggesting that decreases in salivary secretion are not the only mechanism for the periodontal disease experienced by these patients. Subsequently they also hold significant promise for therapeutics either through the manipulation of resident defensin levels or introduction of topicals that work via similar mechanisms. Indeed, defensins upregulation of inflammation may be one potential mechanism by which ultraviolet light works in many cutaneous conditions, as it has been shown that UV light downregulates defensin density on the skin, in part through its induction of vitamin D (Schwalfenberg 2011).

Cross-References

- ▶ Normal Immune Function and Barrier: Epithelial Barrier
- ▶ Normal Immune Function and Barrier: Gamma Delta T-Cells
- ▶ Normal Immune Function and Barrier: Langerhans Cells

References

Bolognia J, Jorizzo JL, Schaffer JV. Dermatology, vol. 2. Philadelphia: Elsevier Saunders; 2012.

Carvalho Ade O, Gomes VM. Plant defensins and defensin-like peptides – biological activities and biotechnological applications. Curr Pharm Des. 2011;17(38):4270–93.

Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003;3(9):710–20.

Kaushansky K, Williams WJ. Williams hematology. New York/London: McGraw-Hill Medical/McGraw-Hill [distributor]; 2010.

Levinson W. Review of medical microbiology and immunology. London: Lange Medical Books/McGraw-Hill; 2006.

Niyonsaba F, Nagaoka I, Ogawa H. Human defensins and cathelicidins in the skin: beyond direct antimicrobial properties. Crit Rev Immunol. 2006;26(6):545–76.

Oppenheim JJ, et al. Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. Ann Rheum Dis. 2003;62 Suppl 2:ii17–21.

Peluso G, et al. Proteomic study of salivary peptides and proteins in patients with Sjogren's syndrome before and after pilocarpine treatment. Arthritis Rheum. 2007;56(7):2216–22.

Rich RR. Clinical immunology: principles and practice. Oxford: Elsevier; 2013.

Schauber J, Gallo RL. Antimicrobial peptides and the skin immune defense system. J Allergy Clin Immunol. 2009;124(3 Suppl 2):R13–8.

Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. Mol Nutr Food Res. 2011;55(1):96–108.

White SH, Wimley WC, Selsted ME. Structure, function, and membrane integration of defensins. Curr Opin Struct Biol. 1995;5(4):521–7.

Wolff K, Fitzpatrick TB. Fitzpatrick's dermatology in general medicine, vol. 2. New York/London: McGraw-Hill; 2008.
Yang D, et al. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science. 1999;286(5439):525–8.
Zeya HI, Spitznagel JK. Antibacterial and enzymic basic proteins from leukocyte lysosomes: separation and identification. Science. 1963;142(3595):1085–7.
Zhu S, Gao B. Evolutionary origin of beta-defensins. Dev Comp Immunol. 2013;39(1–2):79–84.

Normal Immune Function and Barrier: Epithelial Barrier

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Synonyms

Enteric epithelium; Epidermis; Epithelial barrier; Epithelium; Gastric mucosa; Mucosa; Skin

Definition

The epithelial barrier is the physical barrier that prevents exogenous substances, whether bacterial, fungal, viral, or just toxic, from entering into the body. In addition, the epithelial barrier of the skin must also serve the specialized function of protecting against dehydration and heat loss. This is achieved in a number of ways both passive, such as impermeability, and by the secretion of antimicrobial peptides such as defensins and cathelicidins which are dealt with elsewhere, active recruitment of inflammatory cells by the resident epithelial cells, and active protection from the epithelial cells themselves (Abbas et al. 2012). Epithelium exists on all areas where an interface between the internal and external organisms exists. The most obvious of these is the skin, but epithelial barriers also line other organs which process or form a barrier to external substances such as the lung and gastrointestinal tract. As such they form an essential part of the innate and adaptive immune systems.

Types of Epithelial Barrier

Given the variety of locations and functions served, there exist several classes of epithelia designed for each purpose. In most internal organs epithelia do serve as a barrier but also must allow absorption of substances and so consist of simple epithelia. As protection, rather than absorption, becomes more and more prominent, so the layer of epithelium tends to thicken. In the esophagus and buccal mucosa, there is stratified squamous epithelia; in the gingival and hard palate, there are parakeratinizing stratified squamous epithelia; and on the epidermis, there is orthokeratinizing stratified squamous epithelia (Bolognia et al. 2012). In this entry we focus on the latter of these and include discussion of the external cornified layer of the skin, a layer that is unique to the skin, essential in its function, and for a long time, poorly understood.

The External Cornified Layer

The skin is composed of stratified squamous epithelium. Keratinocytes are continuously turned over from the basal layer and mature as they rise through the epidermis. As they reach the outermost layer, they undergo terminal differentiation forming mechanically resilient cornified cells (termed corneocytes) (McKee 1996). Initially these layers were thought of as "dead" cells as they did not differentiate further and had lost all organelles including the cell nucleus. However, while these cells lack the power to further evolve, they still form part of a rich and complex environment which we have only recently begun to appreciate. The outer layer of these cells is composed of an insoluble proteinaceous layer termed the cornified envelope. This insolubility is currently believed to be due to both disulfide bonding and extensive cross-linking by N^e-(gamma-glutamyl)lysineisopeptide bonds

between transglutaminases (Kalinin et al. 2002). While this layer account for much of the stratum corneum's physical resilience, equally important is its impermeability. This is largely contributed by a monomolecular layer of ceramides that is covalently bound to the cornified envelope, forming a moisture-resistant lipid envelope. This lipid envelope prevents maceration of the proteinaceous layer, but impermeability is aided by intercellular lipid lamellae made up of ceramides, free fatty acids, cholesterol, and its esters. These low water content, acidic pH, and a resident microflora (Moens and Veldhoen 2012) all contribute to the barrier against colonization and invasion by pathogens (Elias 2007).

In addition to the physical barriers, epithelia also possess a complex immunologic defense system against exogenous agents (Rich and ebrary Inc. 2013). Keratinocytes and gastric epithelia secrete, both passively and in response to stimuli, proteins such as defensins and cathelicidins, antimicrobial peptides, with broad activity, disrupting cell membranes of both gram-positive and gram-negative bacteria, fungi, and viral envelopes. In addition to being able to recruit inflammatory cells from the circulation, the epithelia also host resident lymphocytes, dendritic cells, and natural killer cells which form part of the permanent defense system of this barrier (Wolff and Fitzpatrick 2008). These are each treated in their own sections and will not be dealt with further here.

Keratinocytes and Tight Junctions

Keratinocytes, the precursors of the cornified layer in the skin, not only provide a source of stratum corneum but also help secrete much of the lipid envelope. In addition keratinocytes secrete defensins and chemoattractants in times of wounding, infection, or inflammation and maintain a complex balance between defense and homeostasis, allowing a partially protective bacterial flora to coexist (Schroder 2010). Keratinocytes also produce structural proteins, named keratins, which form filaments that bind the tissue together ensuring cell and tissue integrity (Presland and Jurevic 2002). In addition to these filaments, keratinocytes exhibit two types of cell adhesion structures: desmosomes function to connect keratinocytes to one another whereas hemidesmosomes attach the basal keratinocytes to the basement membrane. Desmosomes are transmembrane proteins with five extracellular domains and calcium-binding motifs that bind their extracellular domains, made of cell adhesion proteins, such as desmoglein and desmocollin, via homophilic binding to the extracellular domains of neighboring cells (Presland and Dale 2000). In addition the skin exhibits adherens and tight junctions which form even tighter intercellular contacts that seal the space between cells separating tissue compartments (Sapra et al. 2012).

Cross-References

- ▶ Normal Immune Function and Barrier: Defensins
- ▶ Normal Immune Function and Barrier: Gamma Delta T-Cells
- ▶ Normal Immune Function and Barrier: Langerhans Cells

References

Abbas AK, Lichtman AH, Pillai S. Cellular and molecular immunology. Philadelphia: Saunders/Elsevier; 2012.

Bolognia J, Jorizzo JL, Schaffer JV. Dermatology, vol. 2. Philadelphia: Elsevier Saunders; 2012.

Elias PM. The skin barrier as an innate immune element. Semin Immunopathol. 2007;29(1):3–14.

Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: assembly and structural features of the cornified cell envelope. Bioessays. 2002;24(9):789–800.

McKee PH. Pathology of the skin: with clinical correlations. London: Mosby-Wolfe; 1996.

Moens E, Veldhoen M. Epithelial barrier biology: good fences make good neighbours. Immunology. 2012;135(1):1–8.

Presland RB, Dale BA. Epithelial structural proteins of the skin and oral cavity: function in health and disease. Crit Rev Oral Biol Med. 2000;11(4):383–408.

Presland RB, Jurevic RJ. Making sense of the epithelial barrier: what molecular biology and genetics tell us about the functions of oral mucosal and epidermal tissues. J Dent Educ. 2002;66(4):564–74.
Rich RR(ed,). Clinical immunology principles and practice. In: Expert consult. London: Elsevier Saunders; 2013. pp. xxvii, 1295 p.
Sapra B, Jindal M, Tiwary AK. Tight junctions in skin: new perspectives. Ther Deliv. 2012;3(11):1297–327.
Schroder JM. The role of keratinocytes in defense against infection. Curr Opin Infect Dis. 2010;23(2):106–10.
Wolff K, Fitzpatrick TB. Fitzpatrick's dermatology in general medicine, vol. 2. New York/London: McGraw-Hill; 2008.

Normal Immune Function and Barrier: Gamma Delta T-Cells

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Synonyms

Dendritic epidermal T-cells; Gamma delta T-cells; γδ-T-cells

Definition

T-cells express T-cell receptors (TCR) on their surface. Although the majority express alpha and beta heterodimer TCRs, γδ-T-cells express gamma and delta chains instead (Bolognia et al. 2012). These γδ-T-cells are prominent in epithelial tissues including the skin, gut, and lung and under normal conditions make up 10 % (Kaushansky and Williams 2010) of all T-cells in these tissues, although in leprosy or leishmaniasis this figure can increase to 33 %. Our understanding of γδ-T-cells continues to expand as new subtypes are identified. γδ-T-cells form a highly heterogeneous population of cells with a variety of physiological roles (Pang et al. 2012). In addition they are thought to remain partially activated at all times, and as such they form an essential part of the rapid response of both the cutaneous adaptive and innate immune systems (Rich 2013).

Differences Between αβ-T-Cells and γδ-T-Cells

There exist a number of differences between the more ubiquitous αβ-T-cells and the γδ-T-cell subset. In contrast to αβ-T-cells which are generally categorized as CD4+ or CD8+, γδ-T-cells are usually both CD4- and CD8-. While αβ-T-cell activation is regulated by MHC class I and II molecules, the activation of γδ-T-cells is not, using instead the junctional adhesion molecule like protein, and the coxsackie- and adenovirus receptor as co-stimulatory molecules. Diversity in the TCR loci is determined by recombination of the variable (V), diversity (D; for β and δ chains), and junctional (J) region sequence elements (Murphy et al. 2012). While in αβ-T-cells in general and γδ-T-cells in the spleen, this leads to a great variation in TCRs, γδ-T-cells in epithelia have much more restricted TCR diversity (Thedrez et al. 2007). While γδ-T-cells can adapt, this response to a more limited, preprogrammed set of antigens makes them also like the innate immune response.

It is likely that γδ-T-cells also play a very different role to αβ-T-cells. γδ-T-cells appear to be responsible for self-regulation and destruction of epithelial cells that have become transformed virally or by malignant transformation (Girardi et al. 2001), metabolically dysregulated, or otherwise stressed. In addition, in the skin, dendritic epidermal T-cells (DETCs), in a manner more similar to Langerhans cells than other T-cells, form a dendritic network in the suprabasal epidermis (Girardi 2006), where they have a role in the promotion of wound healing (Jameson et al. 2004). Finally there is some suggestion that αβ-T-cells may in part be downregulated by γδ-T-cell with γδ-T-cells serving to prevent

excessive inflammatory responses, and that because of their role in dampening immune responses and their regulation of damaged self-cells, they may play a role in autoimmune diseases such as multiple sclerosis (Blink and Miller 2009).

γδ-T-Cells in Infection

γδ-T-cells have been shown to proliferate in a wide variety of infectious states including tularemia, salmonellosis, brucellosis, legionellosis, listeriosis blood, *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, meningitis, ehrlichiosis, Coxiella burnetii, Q-fever, Chlamydia psittaci, malaria, toxoplasmosis, leishmaniasis, *M. tuberculosis*, *M. avium*, and leprosy (Chen and Letvin 2003). γδ-T-cells appear to fulfil multiple roles in infection. First they are recognized to have cytotoxic effects when stimulated by isopentenyl pyrophosphate and alkylamines, compounds found in plants and bacteria (Girardi 2006). Second through secretion of IFN-γ, they may stimulate NK, NKT, and αβ-T-cells. Finally they serve as antigen-presenting cells to αβ-T-cells.

γδ-T-Cells in Malignancy

Just as in infection, in malignancy surveillance γδ-T-cells are believed to have effects via direct cytotoxic mechanisms and induction of cytokines and downregulate a subset of tumor-promoting αβ-T-cells (Marquez-Medina et al. 2012). Like NK cells and αβ-T-cells, γδ-T-cells express NKG2D which in humans binds MHC class I chain-related A and B (MICA and MICB). These are expressed on a variety of tumor cells suggesting an antineoplastic role for γδ-T-cells. In addition, γδ-T-cell-deficient mice are shown to have lower levels of IFN-γ, a cytokine known to be important in tumor immunity, as well as higher rates of tumor development, suggesting a second, cytokine-driven, mechanism for neoplasm surveillance in γδ-T-cells (Gao et al. 2003). As a result a variety of γδ-T-cell-based antineoplastic therapies are currently under investigation.

γδ-T-Cells in Downregulation of the Inflammatory Response

γδ-T-cells are thought to also have a role parallel to that of T-regs. In infection and autoimmunity they have been shown to produce anti-inflammatory cytokines such as interleukin-2 (IL-2), IL-4, interferon-γ (IFN-γ), IL-10, and transforming growth factor-β (TGF-β) (Mukasa et al. 1998). In γδ-T-cells knockout mice skin inflammation is spontaneous, and models of inflammatory skin conditions notably are worse. Finally, a number of other anti-inflammatory properties have been associated with these cells including expression of Fas ligand and expression of lymphoid thymosin-β 4 which has been shown to be anti-inflammatory (Girardi 2006).

Cross-References

- ▶ Normal Immune Function and Barrier: Epithelial Barrier
- ▶ Normal Immune Function and Barrier: Langerhans Cells

References

Blink SE, Miller SD. The contribution of gammadelta T cells to the pathogenesis of EAE and MS. Curr Mol Med. 2009;9(1):15–22.

Bolognia J, Jorizzo JL, Schaffer JV. Dermatology, vol. 2. Philadelphia: Elsevier Saunders; 2012.

Chen ZW, Letvin NL. Vgamma2Vdelta2+ T cells and anti-microbial immune responses. Microbes Infect. 2003;5(6):491–8.

Gao Y, et al. Gamma delta T cells provide an early source of interferon gamma in tumor immunity. J Exp Med. 2003;198(3):433–42.

Girardi M. Immunosurveillance and immunoregulation by gammadelta T cells. J Invest Dermatol. 2006;126(1):25–31.

Girardi M, et al. Regulation of cutaneous malignancy by gammadelta T cells. Science. 2001;294(5542):605–9.

Jameson JM, et al. Regulation of skin cell homeostasis by gamma delta T cells. Front Biosci. 2004;9:2640–51.

Kaushansky K, Williams WJ. Williams hematology. New York/London: McGraw-Hill Medical/McGraw-Hill [distributor]; 2010.

Marquez-Medina D, Salla-Fortuny J, Salud-Salvia A. Role of gamma-delta T-cells in cancer: another opening door to immunotherapy. Clin Transl Oncol. 2012;14(12):891–5.
Mukasa A, et al. Gamma delta T cells in infection-induced and autoimmune-induced testicular inflammation. Immunology. 1998;95(3):395–401.
Murphy K, et al. Janeway's immunobiology. New York: Garland Science; 2012.
Pang DJ, et al. Understanding the complexity of gammadelta T-cell subsets in mouse and human. Immunology. 2012;136(3):283–90.
Rich RR. Clinical immunology: principles and practice. Oxford: Elsevier; 2013.
Thedrez A, et al. Self/non-self discrimination by human gammadelta T cells: simple solutions for a complex issue? Immunol Rev. 2007;215:123–35.

Normal Immune Function and Barrier: Langerhans Cells

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Synonyms

Antigen-presenting cell; Dendritic cell; Langerhans cell

Definition

Langerhans cells (LCs) are the primary antigen-presenting cells of the skin. As such they form an essential part of the cutaneous adaptive immune system.

Historical Background

Langerhans cells were named after Paul Langerhans who first described these cells in 1868 (Langerhans 1886). Initially believed to be neural in origin due to their dendritic form, it was only much later that they were identified as originating in bone marrow and being in the general category of antigen-presenting cells (Goldsmith and Fitzpatrick 2012).

Histology

Langerhans cells are present in the skin and oral mucosa. While they can be found in any layer in normal skin, they are at greatest concentration in the stratum spinosum (Young 2006), although their location is more variable in mucosal surfaces (Upadhyay et al. 2013). LCs can also be subdivided by type. Type 1 LCs have more dendrites, a lucent cytoplasm on electron microscopy, and numerous granules and are more often located in the suprabasal layers. In contrast, type 2 LCs have less dendrites, an electron-dense cytoplasm, and fewer Birbeck granules. Type 2 LCs are found in the basal layer. LCs account for 25 % of the skin's surface area (Upadhyay et al. 2013), having from 5 to 9 dendrites in the plane of the skin, although these decrease in density with age (Weedon and Strutton 2002). They are not visible on normal hematoxylin and eosinophil-stained sections but can be seen on electron microscopy or histochemical sections. While a variety of markers, such as ATPase, CD45, peanut lectin, S100, vimentin, and HLA-DR, can be used to highlight LCs, CD1a is more commonly used as within the epidermis it is unique to LCs (Weedon and Strutton 2002). The characteristic structural feature of LCs is the Birbeck granules which on electron microscopy are visible as rod-shaped endosomal organelles that functionally are responsible for both antigen capture and presentation (Collin et al. 2013).

Function

LCs are important in both the sensitization and effector phases of the adaptive immune response (Henri et al. 2010; Rich 2013; Zaba et al. 2009). Initially LCs remain quiescent in the skin until stimulated by exposure to microbial or nonmicrobial antigens. In this immature state, LCs express a variety of surface receptors

including Toll-like receptors, C-type lectin receptors, Fc-g, and complement receptors (Cutler and Jotwani 2004). Upon first exposure to a new antigen, LCs change from high E-cadherin to low E-cadherin levels, reducing their adherence and allowing them to migrate out of the epidermis. Activated LCs also express CCR7, a cell surface receptor that is specific for chemotactating cytokine produced in the T-cell zone of lymph nodes, and they therefore home to these upon entering the afferent lymphatics (Rich 2013). These activated LCs then present the antigen to naïve T cells in the draining lymph nodes, causing T-cell differentiation (Igyarto and Kaplan 2013). A subset of these memory T cells migrate to the skin and remain dormant until stimulated in the effector phase, and some remain in regional lymph nodes. During the effector phase, prior exposure has already occurred, and in this case, LCs first activate tissue-resident memory T cells that have been pre-primed to the antigen to become T-effector cells. In addition, some activated LCs migrate to the draining lymph nodes where they can again present the antigen and activate resident memory T cells to become skin homing for increased response. While initially LCs' primary role was believed to be in the adaptive response to microbial antigens, increasing evidence suggests that they have a multiplicity of roles and may be equally important in responding to cutaneous malignancy (Yanofsky et al. 2013). The presentation of exogenous antigen-derived peptides occurs primarily via the MHC class II presentation to CD4 + helper T cells. However, LCs have also been shown to play a role in MHC class I restricted cytotoxic (CD8+) T-cell responses, as well as stimulating NK cells via production of IL-12. In addition, they have a significant stimulatory role via cytokine production including IL-α, IL-β, IL-6, IL-7, IL-12, IL-15, IL-18, TNF-α, TGF-β, M-CSF, and GM-CSF (Upadhyay et al. 2013).

Disease States

In addition to the normal homeostasis and immunity within the skin, LCs have been shown to have a dominant role in a number of cutaneous and mucosal disease states including atopic dermatitis (Bolognia et al. 2012; Novak 2012) and lichen planus (Sugerman et al. 2002), as well as being deranged in graft versus host disease and HIV, the latter of which is primarily thought of as a CD4 T-cell disease but which has prominent skin findings associated with its effect on LCs. LCs are also indicated in a number of diseases where the primary cell is the LC such as Langerhans cell histiocytosis. Subsequently they also hold significant promise for induction of immune response in therapy, where this is desired such as in administration of vaccines (Teunissen et al. 2012) and antineoplastic therapies (Yanofsky et al. 2013).

Cross-References

- ▶ Normal Immune Function and Barrier: Defensins
- ▶ Normal Immune Function and Barrier: Epithelial Barrier
- ▶ Normal Immune Function and Barrier: Gamma Delta T-Cells

References

Bolognia J, Jorizzo JL, Schaffer JV. Dermatology, vol. 2. Philadelphia: Elsevier Saunders; 2012.

Collin M, McGovern N, Haniffa M. Human dendritic cell subsets. Immunology. 2013;140(1):22–30.

Cutler CW, Jotwani R. Antigen-presentation and the role of dendritic cells in periodontitis. Periodontol 2000. 2004;2000(35):135–57.

Goldsmith LA, Fitzpatrick TB. Fitzpatrick's dermatology in general medicine. New York: McGraw-Hill Professional; 2012.

Henri S, et al. Disentangling the complexity of the skin dendritic cell network. Immunol Cell Biol. 2010;88(4):366–75.

Igyarto BZ, Kaplan DH. Antigen presentation by Langerhans cells. Curr Opin Immunol. 2013;25(1):115–9.

Langerhans P. Nerven der menschlichen Haut. Virchows. Arch Abt B Pathol Anat. 1886;44:325–37.

Novak N. An update on the role of human dendritic cells in patients with atopic dermatitis. J Allergy Clin Immunol. 2012;129(4):879–86.

Rich RR. Clinical immunology: principles and practice. Oxford: Elsevier; 2013.
Sugerman PB, et al. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med. 2002;13(4):350–65.
Teunissen MB, Haniffa M, Collin MP. Insight into the immunobiology of human skin and functional specialization of skin dendritic cell subsets to innovate intradermal vaccination design. Curr Top Microbiol Immunol. 2012;351:25–76.
Upadhyay J, et al. Langerhans cells and their role in oral mucosal diseases. N Am J Med Sci. 2013;5(9):505–14.
Weedon D, Strutton G. Skin pathology. Edinburgh: Churchill Livingstone; 2002.
Yanofsky VR, et al. Understanding dendritic cells and their role in cutaneous carcinoma and cancer immunotherapy. Clin Dev Immunol. 2013;2013:624123.
Young B. Wheater's functional histology: a text and colour atlas. Edinburgh: Churchill Livingstone/Elsevier; 2006.
Zaba LC, Krueger JG, Lowes MA. Resident and "inflammatory" dendritic cells in human skin. J Invest Dermatol. 2009;129(2):302–8.

Normal Immune Function and Barrier: Vitamin D

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Synonyms

1,25(OH)2D3; 1,25-Dihydroxyvitamin D (1,25(OH)2D3); 22-Dihydroergocalciferol; 25(OH)D3; 25-Hydroxycholecalciferol; 25-Hydroxyvitamin D; Calcidiol; Calcifediol; Calcitriol; Cholecalciferol; Ergocalciferol; Sitocalciferol; Vitamin D; Vitamin D2; Vitamin D3; Vitamin D4; Vitamin D5

Definition

Vitamin D is not one molecule, but a family of molecules, all of which are in the category of secosteroids, which are steroids in which one of the bonds in the steroid rings is broken. In human health, the two most important molecules in the family of vitamin D are currently believed to be vitamin D3, or cholecalciferol, and vitamin D2, or ergocalciferol, and most commonly when discussing vitamin D, it is in reference to one or both of these molecules. However, in total there are five vitamin D molecules including vitamin D1, ergocalciferol with lumisterol; vitamin D2, ergocalciferol; vitamin D3, cholecalciferol; vitamin D4, 22-dihydroergocalciferol; and vitamin D5, sitocalciferol (Harrison and Longo 2013).

Sources and Biosynthesis of Vitamin D

Both vitamin D2 and vitamin D3 can be ingested in the diet. In the skin, ultraviolet light at a wavelength of 280–320 nM converts 7-dehydrocholesterol to provitamin D3, which is then rapidly converted to vitamin D3 at the plasma membrane (Fitzpatrick and Wolff 2008; Holick et al. 2007). Vitamin D3 is then converted to calcidiol (25-hydroxycholecalciferol). Vitamin D2 (ergocalciferol) is converted in the liver to 25-hydroxyergocalciferol. These metabolites are the molecules measured in serum to determine if a patient is vitamin D deficient. The majority of metabolically active vitamin D is then produced by further breakdown in the kidneys to form 1,25 dihydroxyvitamin D ($1,25(OH)_2D$). However, a small amount of 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) is also produced locally in the skin as keratinocytes also possess small quantities of the relevant enzymes (CYP27A1, the 25-hydroxylase, and CYP27 B1, the 1-hydroxylase) (Bikle 2012).

Vitamin D and Structure and Integrity of the Epithelial Barrier

Vitamin D plays a role in the structure and function of a number of essential components of the epithelial barrier. Adherens junctions are complexes of E-cadherin and nectin and cytosolic scaffolds, α-catenin, β-catenin, and p120-catenin in the plasma membrane of epithelial cells which allow intercellular signaling and help bind cells of the epithelial barrier together

(Hartsock and Nelson 2008). In particular the β-catenin pathway has been identified as contributing to both homeostasis and neoplastic surveillance and is in part regulated by activation of the vitamin D receptor (VDR) (Campbell et al. 1997; Leyssens et al. 2013).

Similar to adherens tight junctions are important in epithelial barrier, but serve to prevent passage across the barrier between cells and maintain homoeostasis (Niessen 2007). One of the most well-established roles of vitamin D is in calcium homeostasis and signaling. It has been shown that claudin-2 and 12 molecules within the tight junction are important co-regulators in this vitamin D-controlled regulation across epithelial barriers (Sun 2010) and that conversely tight junction function can be hampered by downregulation of occludin, ZO-1 and claudin 2, 10b, 12 and 15 in vitamin D deficiency (Hwang et al. 2013; Yin et al. 2011). In addition, dysregulation of vitamin D-mediated calcium signaling leads to poor differentiation in keratinocytes.

While calcium-starved keratinocytes do proliferate, they do so at a reduced rate; in addition, they do not stratify or form strong intercellular contacts, and fail to produce cornified envelopes an essential part of the integrity of the epithelial barrier (Bikle 2012).

Vitamin D and the Immune Defense of the Normal Epithelial Barrier

Vitamin D is believed to play an important role in homoeostasis as $1,25(OH)_2D3$ both supports maintenance of innate surveillance and suppresses the adaptive response (Hart et al. 2011). Immature macrophages and dendritic cells within the epithelial barrier, as well as circulating cells which can home to the barrier express higher levels of VDR. Stimulation of the VDR suppresses the adaptive response of these cells, and in mature, activated cells, VDR production is suppressed, allowing them to function normally.

In addition to this $1,25(OH)_2D3$ induces monocytes, macrophages, neutrophils, and epithelial cells to secrete antimicrobial peptides, such as defensins and cathelicidins, which also make up an essential component of the innate immune response of epithelia (Hewison 2011). In addition to its effect on dendritic cells, the primary antigen-presenting cell in the epithelia, vitamin D3 promotes T_{Reg} cell development while simultaneously suppressing T_H1 and T_H17 cells (Baeke et al. 2010; Hart et al. 2011). Vitamin D3 also suppresses differentiation of B cells into memory and plasma B cell subtypes and antibody production. Finally vitamin D3 appears to modulate both B and T cells homing to the skin and other epithelial barriers. Despite the above studies however, and the clear role for vitamin D in normal epithelial barrier and immune response, studies of the relation of vitamin D to disease states have been variable. In addition, while this has been widely studied, the evidence to support therapeutic value in interventional vitamin D is much weaker although topical vitamin D analogs are common in, e.g., psoriasis and eczema. This suggests that some variation in vitamin D levels in disease states may be a result of the disease process and should not be confused with the hypothesis, which to date remains unsupported by the available scientific data, that vitamin D is instead the prime mechanism of disease production (Autier and Gandini 2007).

Cross-References

- ▶ Normal Immune Function and Barrier: Defensins
- ▶ Normal Immune Function and Barrier: Epithelial Barrier
- ▶ Normal Immune Function and Barrier: Gamma Delta T-Cells
- ▶ Normal Immune Function and Barrier: Langerhans Cells

References

Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med. 2007;167(16):1730–7.

Baeke F, et al. Vitamin D: modulator of the immune system. Curr Opin Pharmacol. 2010;10(4):482–96.

Bikle DD. Vitamin D and the skin: physiology and pathophysiology. Rev Endocr Metab Disord. 2012;13(1):3–19.
Campbell MJ, et al. Inhibition of proliferation of prostate cancer cells by a 19-nor-hexafluoride vitamin D3 analogue involves the induction of p21waf1, p27kip1 and E-cadherin. J Mol Endocrinol. 1997;19(1):15–27.
Fitzpatrick TB, Wolff K. Fitzpatrick's dermatology in general medicine, vol. 2. New York: McGraw-Hill Medical; 2008.
Harrison TR, Longo DL. Harrison's manual of medicine. New York: McGraw-Hill Medical; 2013.
Hart PH, Gorman S, Finlay-Jones JJ. Modulation of the immune system by UV radiation: more than just the effects of vitamin D? Nat Rev Immunol. 2011;11(9):584–96.
Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. Biochim Biophys Acta. 2008;1778(3):660–9.
Hewison M. Antibacterial effects of vitamin D. Nat Rev Endocrinol. 2011;7(6):337–45.
Holick MF, et al. Vitamin D and skin physiology: a D-lightful story. J Bone Miner Res. 2007;22 Suppl 2:V28–33.
Hwang I, et al. Alteration of tight junction gene expression by calcium- and vitamin d-deficient diet in the duodenum of calbindin-null mice. Int J Mol Sci. 2013;14(11):22997–3010.
Leyssens C, Verlinden L, Verstuyf A. Antineoplastic effects of 1,25(OH)2D3 and its analogs in breast, prostate and colorectal cancer. Endocr Relat Cancer. 2013;20(2):R31–47.
Niessen CM. Tight junctions/adherens junctions: basic structure and function. J Invest Dermatol. 2007;127(11):2525–32.
Sun J. Vitamin D and mucosal immune function. Curr Opin Gastroenterol. 2010;26(6):591–5.
Yin Z, et al. Vitamin D enhances corneal epithelial barrier function. Invest Ophthalmol Vis Sci. 2011;52(10):7359–64.

Novel Targets in Systemic Lupus Erythematosus

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Synonyms

Biologics; Small molecule inhibitors

Definition

Treatment of autoimmune diseases such as systemic lupus erythematosus invariably requires the use of immunosuppressive medications. Better understanding of the pathophysiology of these diseases has allowed the identification of new selective targets for therapeutic intervention.

Historical Background

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease associated with significant morbidity and mortality. The pathogenesis of SLE involves multiple levels of immune dysfunction, as well as multiple organ systems, and therefore, therapy has traditionally required broad-based immunosuppression. Corticosteroids in particular are the mainstay of SLE treatment. Though effective, use of steroids is limited by their long-term toxicities. Other therapies such as cyclophosphamide, mycophenolate mofetil, and azathioprine have markedly improved patient outcomes over the past 20 years. However, these agents still carry risks of toxic side effects, secondary malignancies, and opportunistic infection.

Recently, much effort has been spent in developing newer, targeted therapies that theoretically carry fewer risks and are less immunosuppressive. It is often said that SLE is a heterogeneous disease, since it can present in myriad different ways in different patients. It is therefore likely that the underlying immune dysfunction and other factors are also quite variable from patient to patient, and the selection of targeted therapy may in future be guided by the individual phenotype.

The pathogenesis of SLE is quite complex and remains incompletely understood (Tsokos 2011). Both innate and adaptive arms of the immune system are dysregulated with regard to clearance of antigen and apoptotic debris, antigen presentation, response to stimulation, and cytokine production. At present, the development of targeted therapies has been focused on immune abnormalities; however, genetic, epigenetic,

environmental, and hormonal influences all play a role in SLE pathogenesis and these areas may represent new areas for therapeutic intervention in future.

Therapy Directed Against B Cells

SLE is characterized by the presence of autoantibodies, which can precede the onset of clinical symptoms by many years. However, only some of these autoantibodies have been shown to play a direct role in pathogenesis. For example, antibodies directed against double-stranded DNA (dsDNA) have been shown to induce inflammation by depositing in the kidney, either as part of immune complexes formed elsewhere or due to cross-reactivity with kidney antigens (Deshmukh et al. 2006). Serum titers of anti-dsDNA antibodies correlate with lupus nephritis disease activity. Similar disease correlations have been proposed for other autoantibodies found in SLE, and the cytopenias in SLE have also been attributed to autoantibody-mediated destruction. Therefore, several different B cell–directed therapies attempting to decrease autoantibody production have been proposed (Table 1).

B Cell Depletion

Rituximab, a chimeric monoclonal antibody directed against CD20, was first developed for the treatment of B cell lymphomas. Its use has subsequently been expanded to include the treatment of a wide variety of autoimmune conditions, including SLE, rheumatoid arthritis, and vasculitis. As CD20 is a surface marker expressed on B cells (through development from pre-B cell precursors to mature and memory B cells), rituximab induces rapid and sometimes long-lasting depletion of circulating B cells. The effect on B cells in the periphery is less quantifiable and probably variable. Notably, antibody-producing plasma cells do not express CD20 and therefore are not affected by rituximab administration. Total serum immunoglobulin levels are also not usually affected. However, levels of autoantibodies such as anti-dsDNA in SLE have been shown to decline after rituximab treatment. Interestingly, rituximab's clinical benefits are often apparent much sooner than detectable changes in autoantibody levels. This discrepancy suggests a mechanism of action beyond inhibition of autoantibodies. In addition to production of autoantibodies, B cells may potentiate SLE pathogenesis through abnormal antigen presentation and costimulation of T cells, setting up a cycle of hyperreactivity. Another hypothesis suggests that clearance of B cells opsonized by rituximab may divert complement and inflammatory cells from sites of immune complex deposition that might otherwise lead to damage. While multiple small case series of SLE patients have described improved outcomes with rituximab, several large, randomized controlled trials have failed to show significant benefit (Lateef and Petri 2010). Use of rituximab for the treatment of SLE, though commonly accepted, remains off-label.

Other B cell–depleting antibodies are also in development. Ocrelizumab and ofatumumab are also antibodies directed against CD20; both bind to different epitopes as compared to rituximab and this binding may allow for more efficient cell killing. Further development of ocrelizumab has been halted due to concern for increased risk of infection. Epratuzumab is a humanized anti-CD22 monoclonal antibody; CD22 is a surface marker expressed on mature B cells. Epratuzumab is currently under phase III investigation for treatment of SLE. Combination therapy with epratuzumab and rituximab, theorized to enhance efficiency of treatment, is also being studied in patients with lymphoma. Antibodies directed against CD19 (expressed through development from pro-B precursors to mature B cells) are also in development (Lateef and Petri 2010). Finally, targeted inhibition of antibody-secreting plasma cells has also been considered. While such agents exist for the treatment of myeloma, detailed studies in patients with SLE have not been published.

B Cell Cytokine Inhibition

Another approach has been to target cytokine signaling in order to inhibit B cell activity and function. Belimumab, a humanized monoclonal

Novel Targets in Systemic Lupus Erythematosus, Table 1 Development of targeted therapies for treatment of SLE

Target	Drug	Mechanism	Development phase
B cell			
CD20	Rituximab	mAb against CD20; depletes mature B cells and B cell precursors	Large phase II/III studies did not meet primary endpoint; commonly used off-label
	Ocrelizumab	mAb against CD20	Phase III halted due to infections; other trials ongoing in RA and multiple sclerosis
	Ofatumumab	mAb against CD20	Trials in lymphoma and leukemia; no studies in SLE
	Veltuzumab	mAb against CD20	Trials in RA, ITP, and lymphoma; no studies in SLE
CD22	Epratuzumab	mAb against CD22; depletes mature B cells	Phase III study in progress
BLyS/BAFF	Belimumab	mAb against BAFF	FDA approved for SLE
	Atacicept	Fusion protein of TACI receptor with Fc	Phase II/III completed, results pending
	Briobacept	Fusion protein of BAFFR with Fc	Preclinical development
B cell tolerogen	Edratide	Peptide derived from human anti-DNA antibody	Phase II did not meet primary endpoint
	Abetimus	Oligonucleotide that binds anti-dsDNA antibodies	Phase III halted for lack of efficacy
T cell			
IL-17	AIN457	mAb against IL-17A	Trials in psoriasis, uveitis, RA; no studies in SLE
IL-23	Ustekinumab	mAb against p40 subunit of IL-12 and IL-23	FDA approved for psoriasis; no studies in SLE
Syk	Fostamatinib	Syk inhibitor	Phase II/III studies for RA; no studies in SLE
Jak	Tofacitinib	Jak1/Jak3 inhibitor	FDA approved for RA; no studies in SLE
	LY3009104	Jak1/Jak2 inhibitor	Phase II studies for RA; no studies in SLE
ROCK	Fasudil	ROCK inhibitor	Phase I for secondary Raynaud's showed no benefit; no studies in SLE
Metabolism	Sirolimus	mTOR inhibitor	Phase II studies in progress
Costimulation			
CD40:CD40L	BG9588	mAb against CD40L	Phase II halted due to thromboembolic complications
	IDEC-131	mAb against CD40L	Phase II did not meet primary endpoint
CD28:B7	Abatacept	Fusion of CTLA-4 with Fc	Phase III in progress
	Belatacept	Fusion of CTLA-4 with Fc	Trials in RA, renal transplant; no studies in SLE
ICOS:B7RP1	AMG 557	mAb against B7RP-1	Phase I studies in progress
Other cytokines			
IL-6	Tocilizumab	mAb against IL-6R	Phase I; neutropenia was common side effect
TNF-α	Infliximab	mAb against TNF-α	Phase II/III terminated for logistical reasons
IFN-α	Rontalizumab	mAb against IFN-α	Phase II in progress
	Sifalimumab	mAb against IFN-α	Phase II in progress
	MEDI-546	mAb against IFNAR	Phase II in progress
IFN-γ	AMG 811	mAb against IFN-γ	Phase I in progress
Other			
ROS	*N*-acetylcysteine	Antioxidant	Phase I/II in progress
Complement	Eculizumab	mAb against C5	Phase I/II terminated for logistical reasons
TLR	DV1179	TLR7 and TLR9 inhibitor	Preclinical development

mAb monoclonal antibody, *Fc* Fc portion of IgG, *RA* rheumatoid arthritis, *ITP* idiopathic thrombocytopenia, *IFNAR* IFN alpha receptor

antibody directed against the cytokine B cell activating factor of the TNF family (BAFF, also known as B lymphocyte stimulator, or BLyS), was approved in 2011 for treatment of SLE. BAFF is widely secreted by monocytes and other myeloid cells and is a key regulator of B cell function and homeostasis. The BAFF receptor (BAFF-R) is expressed on effector T cells and B cells throughout development. BAFF additionally binds to two other B cell receptors, TACI (transmembrane activator and CAML interactor) and, with lesser affinity, BCMA (B cell maturation antigen). TACI signaling has negative regulatory properties; mutations in TACI have been associated with common variable immunodeficiency (CVID) and CVID-related autoimmunity. A BAFF-related cytokine named APRIL (a proliferation inducing ligand) also binds to both TACI and BCMA to mediate distinct but overlapping effects. BCMA has higher affinity for APRIL than for BAFF, and is the predominant receptor on plasma cells. APRIL additionally binds heparan sulfate proteoglycans, which likely function as a co-receptor in part by increasing APRIL's interaction with BCMA and TACI. BAFF and APRIL signaling plays critical roles in promoting B cell survival and influencing responses to antigen, including the germinal center responses, immunoglobulin production, and class switching (Rickert et al. 2011).

Increased levels of BAFF have been described in several autoimmune conditions, and SLE in particular. Depletion of BAFF with belimumab in mouse models of SLE leads to a decrease in circulating mature B cell numbers, with a concomitant decline in activated T cells and inflammatory cytokines. In humans, a large phase III study (BLISS 52) showed higher clinical responses and fewer flares in patients treated with belimumab as compared to placebo. A second phase III study, BLISS 76, showed a similar benefit although the difference was not as apparent. Based on these results, belimumab was the first new drug to receive FDA approval for the treatment of SLE in more than 50 years (Davidson 2010). The BAFF signaling pathway has also been targeted using a receptor fusion protein. Atacicept combines the Fc portion of human IgG with the extracellular domain of TACI, thus inhibiting both BAFF and APRIL signaling. Of note, APRIL/BCMA signaling is important for survival of antibody-producing plasma cells. It is therefore possible that atacicept would have a more significant effect on immunoglobulin levels than belimumab. Indeed, a phase II/III study of atacicept in combination with mycophenolate mofetil in SLE patients was stopped prematurely due to unexpectedly profound drops in immunoglobulin levels (Ginzler et al. 2012; Davidson 2010).

Interleukin-6 (IL-6) is another important cytokine made by cells of both the adaptive and innate immune systems. Among many other pro-inflammatory effects, IL-6 encourages the growth and survival of antibody-producing B cells. IL-6 also influences T cell differentiation by promoting IL-17 production and inhibiting regulatory T cell development. Patients with SLE demonstrate higher levels of IL-6 in serum and, in patients with lupus nephritis, also in urine. IL-6 polymorphisms have also been described in patients with lupus. Tocilizumab, a monoclonal antibody directed against the IL-6 receptor, is approved for the treatment of rheumatoid arthritis. The utility of tocilizumab therapy in lupus remains unknown; a small phase I clinical study showed improvement in disease parameters for most patients, but neutropenia was a limiting side effect (reviewed in Lo and Tsokos 2012).

B Cell Tolerogens

Autoantibodies themselves have also been targeted for therapeutic intervention. Edratide is a synthetic peptide based on the complementarity-determining region of anti-DNA antibodies. In mouse models of lupus, immunization with edratide was capable of inducing tolerance and ameliorating disease. However, human studies were disappointing. Another molecule, abetimus (previously LJP-394), was also designed to target anti-dsDNA antibodies. This molecule is comprised of four double-stranded oligonucleotides that bind anti-dsDNA antibodies, cross-linking Ig molecules that are either free-floating or membrane-bound on B cells. Fixation of

antibody by abetimus on B cell surfaces is not immunostimulatory but rather induces apoptosis of the B cell, producing anti-dsDNA antibodies. Abetimus' mechanism of action as a B cell "tolerogen," therefore, is via depletion of circulating anti-dsDNA antibodies as well as downregulation of anti-dsDNA production itself. While initial trials in human patients with SLE were promising, larger follow-up studies did not show significant benefit with treatment (Lo and Tsokos 2012).

Therapy Directed Against T Cells

Although SLE is often thought of as an autoantibody-mediated disease, T cell function is also markedly altered. T cells from SLE patients show skewed phenotypes and cytokine profiles, and demonstrate intrinsic signaling abnormalities that can be potentially targeted for therapy (Kyttaris and Tsokos 2011).

T Cell Cytokine Inhibition

Th17 cells are a helper T cell subset that secretes IL-17, a cytokine that promotes local inflammation in peripheral tissue through recruitment of inflammatory cells and other mechanisms. More recently, other T cell subtypes (particularly innate immune cells such as TCRγ/δ T cells) have also been shown to secrete IL-17. Th17 cells and IL-17 signaling have been increasingly implicated in the pathogenesis of several autoimmune conditions, including inflammatory bowel disease, multiple sclerosis, and psoriasis (Zhu and Qian 2012). In SLE patients, increased numbers of IL-17-secreting T cells have been found both in affected kidneys as well as in peripheral circulation; serum titers of IL-17 are also increased and correlate with disease activity.

Attempts to inhibit IL-17/IL-17R signaling for therapy of autoimmune disorders have used various approaches targeting upstream and downstream factors. IL-6 is another pro-inflammatory cytokine necessary for the differentiation of Th17 cells; a trial of tocilizumab in SLE patients was associated with neutropenia as described earlier. Th17 development and expansion is also dependent on IL-23, which has been successfully targeted by the monoclonal antibody ustekinumab, approved for treatment of psoriasis (Zhu and Qian 2012). Clinical trials studying ustekinumab in multiple sclerosis and inflammatory bowel disease are currently in progress. AIN457, a monoclonal antibody directed against IL-17A itself, is also in clinical development for treatment of psoriasis and other autoimmune conditions.

T Cell Signaling Inhibition

TCR Signaling

Activation of the T cell receptor (TCR) in SLE T cells induces a hyperactive calcium flux response compared to normal T cells. Despite this, SLE T cells produce less IL-2 and do not proliferate as well in vitro. This discrepancy is not fully understood. The aberrant calcium flux response can be traced back to the TCR complex itself. SLE T cells express lower levels of CD3ζ, which typically signals as part of the surface TCR complex through the intracellular tyrosine kinase zeta-associated protein 70 (ZAP70). CD3ζ is substituted in SLE T cells by FcRγ, a homologous receptor normally expressed as part of the FcεRI, FcαR, FcγRI, and FcγRIII receptor complexes on myeloid and NK cells. FcRγ, in turn, partners with spleen tyrosine kinase (Syk) rather than ZAP70. This signaling pathway through Syk activation leads to much faster and stronger calcium flux responses (Kyttaris and Tsokos 2011). Notably, Syk is expressed in a wide variety of cell types and is particularly critical for B cell receptor signaling. Platelet activation is also mediated in part through Syk. Syk inhibition may thus have far-reaching effects beyond correction of T cell signaling abnormalities; mice with autoimmune diabetes treated with Syk inhibitors showed lower B cell numbers and immunoglobulin levels (Colonna et al. 2010). In lupus-prone mice, inhibition of Syk with fostamatinib (previously known as R788) attenuated disease manifestations (Lo and Tsokos 2012). In humans, Syk inhibitors are also being studied for the treatment of rheumatoid arthritis and other autoimmune

conditions. Fostamatinib, a small molecule inhibitor of Syk, showed significant benefit over placebo in a large phase III study of patients with rheumatoid arthritis, with few side effects (Weinblatt et al. 2010); follow-up studies are ongoing.

Jak/STAT Signaling

The Janus kinases (Jak) and signal transducers and activators of transcription (STAT) proteins partner together in different combinations to mediate cytokine signaling (Zerbini and Lomonte 2012). For example, the IL-2 receptor complex signals through binding of the common gamma chain (γc) receptor to Jak3, which in turn phosphorylates STAT5. Phosphorylated STAT5 forms homodimers and translocates to the nucleus where it directly binds promoter regions to activate gene transcription. Similarly, IL-6 signal transduction pathway is via Jak1 and Jak2, which partner with STAT1 and STAT3 to mediate gene activation. Several different Jak inhibitors are currently under development in the treatment of autoimmune diseases. Tofacitinib is a small molecule kinase inhibitor of Jak1 and Jak3, with weaker effects on Jak2. In vitro studies show that tofacitinib inhibits IL-4-dependent differentiation of Th2 and Th17 cells, among other effects. In humans, tofacitinib induced significant response rates compared placebo in a phase III study of rheumatoid arthritis. Studies of tofacitinib in other autoimmune diseases are ongoing (Zerbini and Lomonte 2012). Other selective Jak inhibitors are also being developed in an effort to avoid over-immunosuppression, since Jak3 is a critical component of all cytokine signaling pathways that utilize the common γc receptor (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21); congenital Jak3 deficiency results in severe combined immunodeficiency. Selective Jak2 inhibition decreases IL-6- and IFNγ-mediated inflammation without affecting IL-2 signaling. This may be especially advantageous in patients with SLE, in whom lower regulatory T cell numbers have been linked to lower levels of IL-2. A Jak2 inhibitor was recently shown to reduce disease manifestations in mouse models of lupus. Other selective Jak inhibitors are also being studied in patients with rheumatoid arthritis (Lu et al. 2011).

Calcium Signaling

As described above, TCR activation leads to rapid influx of calcium that activates calcineurin. This calcium flux response is increased in SLE T cells. Calcineurin is a phosphatase that dephosphorylates the transcription factor NFAT, allowing it to translocate to the nucleus and mediate cytokine gene expression. Thus, the calcineurin inhibitors cyclosporine and tacrolimus act primarily by inhibiting T cell cytokine production. Cyclosporine and tacrolimus are sometimes given as second-line agents in the treatment of lupus, particularly lupus nephritis, but their use is limited by renal toxicity. Recently, dipyridamole, an inhibitor of platelet aggregation, was also found to inhibit calcineurin activity in vitro, and it attenuated disease activity in mouse models of lupus (Kyttaris and Tsokos 2011).

Rho Kinase

Tissue damage in SLE is due in part to increased migration of inflammatory cells to end organs. T cells in SLE patients express higher levels of surface markers such as chemokine receptors and CD44, a glycoprotein that binds hyaluronic acid. CD44+ cells have been found infiltrating the kidneys of patients with lupus nephritis. CD44 binds to a family of proteins (ezrin, radixin, and moesin, collectively known as ERM) that link the cell surface to the intracellular cytoskeleton to promote cell adhesion and migration. The activity of the ERM proteins is dependent on phosphorylation by the rho kinase ROCK, and this activity is increased in SLE T cells. Another rho kinase, ROCK2, activates interferon regulatory factor-4 (IRF-4), a transcription factor important for Th17 differentiation. In mouse models of lupus, fasudil, a ROCK inhibitor, decreased IL-17 and IL-21 production and improve manifestations of disease (Stirzaker et al. 2012). While ROCK inhibitors have not yet been studied in humans with lupus, fasudil also has vasodilatory properties and is currently being investigated for the treatment of Raynaud's phenomenon.

Mitochondrial Dysfunction

The mammalian target of rapamycin (mTOR) is a mitochondrial membrane protein that controls transmembrane potential. Lymphocytes from SLE patients demonstrate abnormal mitochondrial function with hyperpolarization, higher production of reactive oxygen intermediates, increased oxidative stress, and lower ATP content. SLE T cells have normal levels of mTOR expression but show increased mTOR activity. Treatment of SLE T cells in vitro with an mTOR inhibitor (rapamycin, also known as sirolimus, used to prevent organ rejection in transplantation) increased CD3ζ expression and normalized calcium flux responses. In SLE patients treated with rapamycin, similar normalization of T cell signaling was also observed. This effect appears to be unrelated to rapamycin's other immunomodulatory properties (Perl 2010).

Costimulation

The costimulatory interactions between T cells, B cells, and antigen-presenting cells (APC) are critical for the perpetuation of inflammation in autoimmune disease. Naïve T cell activation is strongly enhanced by CD28 interaction with costimulatory molecules B7-1 (CD80) and B7-2 (CD86) on activated APCs; CD28 signaling responses influence T cell cytokine production and differentiation. CD28 is a member of a family of homologous receptors that have different downstream effects, both inflammatory and inhibitory. CTLA-4 is one such homolog expressed on regulatory T cells and activated T cells. CTLA-4 binds to the B7 ligands with even stronger affinity than CD28; this binding induces an inhibitory signal important for T cell tolerance (Abbas et al. 2010). Taking advantage of CTLA-4's strong binding affinity, proteins fusing the CTLA-4 extracellular portion to the Fc portion of immunoglobulin have been developed to disrupt CD28:B7 interaction. Abatacept, the first such protein, is already approved for the treatment of rheumatoid arthritis. A phase IIb study of abatacept in SLE patients showed modest therapeutic effects but did not reach its primary endpoint; a larger study in lupus nephritis is in progress (Lateef and Petri 2010).

CD40L (also known as CD154), expressed on activated T cells, binds to B cells and other APCs expressing CD40. This interaction has pro-inflammatory effects via induction of cytokine expression and upregulation of costimulatory receptors; in B cells, immunoglobulin isotype switching, antibody production, and germinal center formation are all dependent on CD40: CD40L interaction. T cell activation and differentiation is influenced indirectly by these effects on the APC (Abbas et al. 2010). SLE patients show higher levels of CD40L expression on T cells as well as higher soluble CD40L levels in peripheral blood. Monoclonal antibodies designed to block CD40L showed promising effects in mouse models of lupus. However, a clinical trial of anti-CD40L in SLE patients was halted due to unexpected thromboembolic events. More recently, CD40L has also been shown to interact with several different integrins, including GPIIb/IIIa on platelets. The effects of anti-CD40L antibodies on platelet activation and thrombus formation are still unclear (Alaaeddine et al. 2012).

Innate Immune System Targets

There appear to be many different points in the adaptive immune system of patients with SLE where tolerance is breached. However, the central role of innate immune system dysregulation in lupus pathogenesis has also become increasingly apparent. Abnormalities in apoptosis, clearance of apoptotic debris, antigen presentation, and immune complex formation have all been described. The type I interferon pathway in particular is perturbed in SLE.

Classically, the type I interferons (IFNα, made by monocytes and dendritic cells, and IFNβ, made primarily by fibroblasts) mediate the innate immune response to viral infection. Activation of pattern recognition receptors such as the Toll-like receptors (TLRs), which recognize foreign DNA, RNA, and other motifs, leads to local IFN production. The response to IFN occurs via activation of Jak/STAT signaling to induce upregulation of antiviral genes (Abbas et al. 2010). In patients with

lupus, increased IFNα levels in serum correlate with a pattern of upregulated IFN-inducible genes in peripheral blood mononuclear cells, termed the "type 1 interferon signature." TLR9 activation in plasmacytoid dendritic cells by unmethylated CpG-DNA is thought to be primarily responsible for this IFN overactivity. Further, genetic studies in SLE patients have identified risk alleles in multiple genes associated with the IFN pathway, strengthening the importance of IFN dysregulation in lupus pathogenesis. Increased IFNα activity may potentiate stimulation of T cells during antigen presentation and inhibit regulatory T cells. This activity has been targeted using anti-IFNα monoclonal antibodies. In phase I studies, sifalimumab and rontalizumab, both anti-IFNα antibodies currently in development, were able to normalize the interferon signature of SLE patients. Clinical studies of MEDI-546, an anti-IFN receptor antibody, are also underway. Finally, an IFNα vaccine has shown encouraging results in early clinical trials. This novel approach uses inactivated cytokine to induce endogenous anti-IFNα autoantibodies (Lichtman et al. 2012).

Hydroxychloroquine is one of the few medications approved by the FDA for treatment of lupus. The antimalarial medications chloroquine and hydroxychloroquine have long been known to have anti-inflammatory effects, although the mechanisms have only recently been elucidated. Both drugs inhibit acidification of lysosomes, interfering with peptide binding to MHC (thus affecting antigen processing and presentation) as well as nucleic acid activation of TLRs. Antimalarial medications may also directly inhibit TLR activation through mechanisms that are not yet understood (Lafyatis et al. 2006). Newer, more potent TLR inhibitors are in development using oligonucleotides with non-stimulatory DNA sequences to block TLR7 and TLR9 activation.

Dysregulated complement activation is a characteristic feature of SLE. Inherited complement deficiencies (especially C1q and C4) are strongly associated with the development of lupus-like disease, suggesting a protective role for complement proteins. Conversely, SLE patients also show acquired complement deficiencies due to immune complex formation. Deposition of complement-containing immune complexes contributes to inflammation and damage in peripheral tissues such as the kidney. Eculizumab, a humanized monoclonal antibody that binds C5 and inhibits its cleavage to C5a and C5b, significantly reduced renal disease in mouse models of lupus. Informative studies in humans with SLE are still pending, although eculizumab has shown clinical efficacy in trials of paroxysmal nocturnal hemoglobinuria, a disorder characterized by spontaneous complement activation causing hemolysis (Bao and Quigg 2007).

Hormonal Therapy

As with many other autoimmune diseases, SLE disproportionately affects women of childbearing age. This observation has led to intense examination of the role of estrogen and other sex hormones in lupus pathogenesis. Estrogen receptors are present in both T and B cells, and estrogen has a number of described effects in vitro, including altered cytokine expression and increased antibody production. Dehydroepiandrosterone (DHEA), a precursor of sex hormones, declines with age and this decline has been proposed to play a role in immune senescence. In vitro, DHEA appears to enhance immune responses and increases IL-2 production in T cells. DHEA levels were observed to be low in patients with SLE and other autoimmune diseases. A randomized controlled trial of DHEA supplementation in women with SLE showed beneficial effects over placebo (Hazeldine et al. 2010).

Environmental and Epigenetic Influences

Environmental exposures such as UV light, infections, medications, and free radical exposure have all been described to contribute to lupus pathogenesis. Reactive oxygen intermediates (ROI)

produced as a by-product of normal metabolism can, when increased by environmental influences, contribute to DNA damage and lead to abnormal cell death. ROI also affects T cell activation and cytokine production, in part by inhibiting expression of CD3ζ. In mouse models of lupus, the antioxidant *N*-acetylcysteine (NAC) improved survival; studies of NAC in humans with SLE have not yet been published (Perl 2010).

Gene expression is also affected by environmental influences through epigenetic modification. In general, SLE lymphocytes show a global pattern of hypomethylation correlating with increased gene expression, including expression of genes implicated in SLE pathogenesis. For example, hydralazine and procainamide are both medications associated with drug-induced lupus. It is now recognized that this phenomenon is related to demethylation of CpG-DNA. Hydralazine directly inhibits the DNA methyltransferase DNMT1, while procainamide inhibits a kinase pathway necessary for DNMT1 and DNMT3 expression; both medications induce a gene expression pattern in T cells similar to that of SLE T cells. Histone modification patterns are also altered in SLE. Histone acetylation of some genes may have protective effects while others may contribute to the SLE phenotype. Histone deacetylase (HDAC) inhibitors are in development for cancer therapy and, based on animal models of lupus, may also hold potential for the treatment of SLE (Hedrich and Tsokos 2011).

Conclusion

Recent advances in the understanding of lupus pathogenesis have allowed for the opportunity to develop new, targeted therapies that will hopefully carry fewer infectious risks than the broader immunosuppressive medications currently in use. One such example has been belimumab, the first new drug approved by the FDA for the treatment of SLE in over 50 years. The biologic medications carry great promise for the treatment of autoimmune diseases but remain expensive to manufacture and administer. The new small molecule inhibitors have the advantage of easier oral dosing, but potential toxicities are less well understood. The eventual success of the targeted therapies discussed here will depend in part on the development of better biomarkers to delineate individual immune phenotypes, optimizing the specificity of care.

Cross-References

- ▶ B7 and CD28 Families
- ▶ CD40
- ▶ CTLA-4
- ▶ CTLA4-Ig
- ▶ Environment and Autoimmunity
- ▶ Epigenetics in Autoimmunity
- ▶ Interleukin-6
- ▶ Juvenile Diseases: SLE in Children
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Mammalian Target of Rapamycin (mTOR)
- ▶ Sjögren's Syndrome
- ▶ Skin in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Animal Models
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Systemic Lupus Erythematosus, Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver

References

Abbas AK, Lichtman AH, et al. Cellular and molecular immunology. Philadelphia: Saunders/Elsevier; 2010.

Alaaeddine N, Hassan GS, et al. CD154: an immunoinflammatory mediator in systemic lupus erythematosus and rheumatoid arthritis. Clin Dev Immunol. 2012;2012:490148.

Bao L, Quigg RJ. Complement in lupus nephritis: the good, the bad, and the unknown. Semin Nephrol. 2007;27(1):69–80.

Colonna L, Catalano G, et al. Therapeutic targeting of Syk in autoimmune diabetes. J Immunol. 2010;185(3):1532–43.

Davidson A. Targeting BAFF in autoimmunity. Curr Opin Immunol. 2010;22(6):732–9.
Deshmukh US, Bagavant H, et al. Role of anti-DNA antibodies in the pathogenesis of lupus nephritis. Autoimmun Rev. 2006;5(6):414–8.
Ginzler EM, Wax S, et al. Atacicept in combination with MMF and corticosteroids in lupus nephritis: results of a prematurely terminated trial. Arthritis Res Ther. 2012;14(1):R33.
Hazeldine J, Arlt W, et al. Dehydroepiandrosterone as a regulator of immune cell function. J Steroid Biochem Mol Biol. 2010;120(2–3):127–36.
Hedrich CM, Tsokos GC. Epigenetic mechanisms in systemic lupus erythematosus and other autoimmune diseases. Trends Mol Med. 2011;17(12):714–24.
Kyttaris VC, Tsokos GC. Targeting lymphocyte signaling pathways as a therapeutic approach to systemic lupus erythematosus. Curr Opin Rheumatol. 2011;23(5):449–53.
Lafyatis R, York M, et al. Antimalarial agents: closing the gate on Toll-like receptors? Arthritis Rheum. 2006;54(10):3068–70.
Lateef A, Petri M. Biologics in the treatment of systemic lupus erythematosus. Curr Opin Rheumatol. 2010;22(5):504–9.
Lichtman EI, Helfgott SM, et al. Emerging therapies for systemic lupus erythematosus – focus on targeting interferon-alpha. Clin Immunol. 2012;143(3):210–21.
Lo MS, Tsokos GC. Treatment of systemic lupus erythematosus: new advances in targeted therapy. Ann N Y Acad Sci. 2012;1247:138–52.
Lu LD, Stump KL, et al. Depletion of autoreactive plasma cells and treatment of lupus nephritis in mice using CEP-33779, a novel, orally active, selective inhibitor of JAK2. J Immunol. 2011;187(7):3840–53.
Perl A. Systems biology of lupus: mapping the impact of genomic and environmental factors on gene expression signatures, cellular signaling, metabolic pathways, hormonal and cytokine imbalance, and selecting targets for treatment. Autoimmunity. 2010;43(1):32–47.
Rickert RC, Jellusova J, et al. Signaling by the tumor necrosis factor receptor superfamily in B-cell biology and disease. Immunol Rev. 2011;244(1):115–33.
Stirzaker RA, Biswas PS, et al. Administration of fasudil, a ROCK inhibitor, attenuates disease in lupus-prone NZB/WF1 female mice. Lupus. 2012;21(6):656–61.
Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365(22):2110–21.
Weinblatt ME, Kavanaugh A, et al. An oral spleen tyrosine kinase (Syk) inhibitor for rheumatoid arthritis. N Engl J Med. 2010;363(14):1303–12.
Zerbini CA, Lomonte AB. Tofacitinib for the treatment of rheumatoid arthritis. Expert Rev Clin Immunol. 2012;8(4):319–31.
Zhu S, Qian Y. IL-17/IL-17 receptor system in autoimmune disease: mechanisms and therapeutic potential. Clin Sci (Lond). 2012;122(11):487–511.

Nuclear Factor of Activated T Cells (NFAT)

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Synonyms

NFAT; NF-AT; Nuclear factor of activated T cells

Definition

The name nuclear factor of activated T cells (NFAT) identifies a group of transcription factors characterized by a conserved DNA-binding domain, which is homologous to the Rel-like DNA-binding domain of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) proteins. The NFAT family comprises five proteins, four of which are characteristically activated upon dephosphorylation of their amino-terminal region by calcineurin, plus a fifth one, NFAT5/TonEBP, which lacks calcineurin-binding motifs and is regulated by different mechanisms. The calcineurin-dependent NFATs are known as NFAT1 (or NFATc2 or NFATp), NFAT2 (or NFATc1 or NFATc), NFAT3 (or NFATc4), and NFAT4 (or NFATc3 or NFATx). The calcineurin-independent NFAT5 is also known as TonEBP (tonicity enhancer binding protein), referring to its function as a hypertonicity-activated factor.

Historical Background

NFAT was first described in 1988 as a DNA-binding protein present in the nucleus of mitogen-activated T lymphocytes and capable of recognizing particular sequences in the human interleukin (IL)-2 gene enhancer and the long terminal repeat of the human immunodeficiency

virus (HIV LTR) (Shaw et al. 1988). As it was also found that unstimulated lymphocytes already contained NFAT in their cytosol in an inactive state, the protein received the names NFATp, the "p" meaning preexisting, and NFATc, the "c" standing for cytosolic. NFAT translocated to the nucleus in response to T cell receptor stimulation or pharmacological agonists of its signaling and associated with a nuclear partner with which it bound to DNA. The first partner identified for NFAT was the heterodimeric transcription factor Fos/Jun (generally known as activator protein-1, AP-1) (Jain et al. 1992), and other transcription factors have been shown to interact with NFAT proteins onto specific DNA sequences. Importantly, the activity of NFAT was found to be inhibited by the immunosuppressants cyclosporin A and FK506 (also known as tacrolimus) by virtue of its dependence on the calcium-activated phosphatase calcineurin.

The first molecular cloning of an NFAT protein, NFAT1, was described by Rao and colleagues in 1993 (McCaffrey et al. 1993) and was followed by the subsequent identification of other NFATs and their respective isoforms. The latest NFAT identified, in 1999, was the calcineurin-independent factor NFAT5 (Miyakawa et al. 1999; Lopez-Rodriguez et al. 1999).

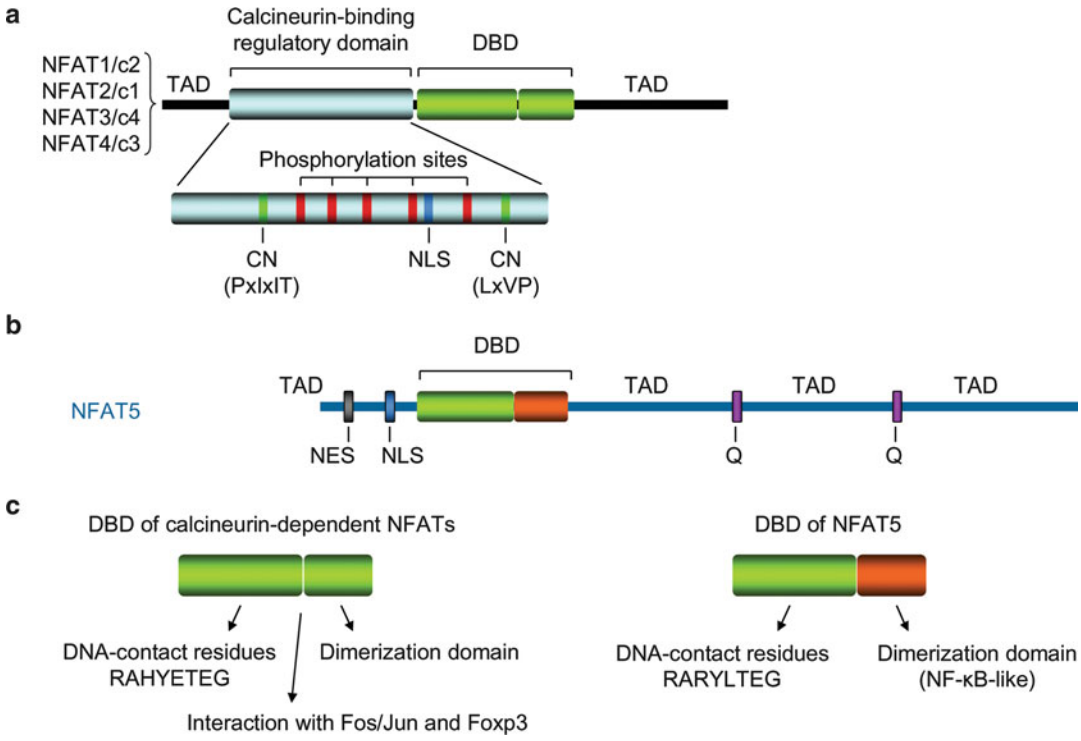
Despite their original name, NFAT proteins are not restricted to T lymphocytes, and they are expressed and functional in different types of immune cells, including B cells, natural killer (NK) cells, neutrophils, macrophages, and megakaryocytes, as well as in nonimmune cells such as neurons, skeletal muscle, cardiomyocytes, endothelial cells, fibroblasts, and adipocytes. Considering the variety of cell types known to use NFAT proteins, it is probably correct to assume that they are present and functional in all nucleated mammalian cell types.

Evolution and Diversity of NFAT Proteins

NFATs are found in vertebrates, and they evolved in two separate branches, one that gave rise to the calcineurin-dependent NFATs and another to NFAT5. Their common Rel-like DNA-binding domain arose from an ancestor that also gave rise to NF-κB proteins (Graef et al. 2001). Factors with a recognizably conserved NFAT-like DNA-binding domain have been described in chordates, such as *Ciona intestinalis*, and in insects, but they lack the calcineurin-binding and phosphorylation-regulated motifs characteristic of the calcineurin-dependent NFATs and are considered to be NFAT5-like proteins. The NFAT found in the fly *Drosophila melanogaster*, known as dNFAT, is capable of conferring resistance to osmotic stress, similarly to NFAT5 in vertebrates. NFATs are not expressed in plants and other commonly studied eukaryotic model organisms, such as yeast and the worm *Caenorhabditis elegans*.

Shared and Distinct Features in the Regulation and Function of NFAT Proteins

Calcineurin-Dependent NFAT Proteins. The calcineurin-dependent NFATs (NFAT1/NFATc2, NFAT2/NFATc1, NFAT3/NFATc4, NFAT4/NFATc3) are encoded in separate genes and contain a well-conserved amino-terminal regulatory region of about 400 amino acids that comprises the nuclear import and export signals and the calcineurin-docking and phosphorylation-regulated sites involved in controlling their nucleocytoplasmic localization (Fig. 1). The two calcineurin-docking motifs of these NFATs contain the conserved amino acid sequences "proline-x-isoleucine-x-isoleucine-threonine" (PxIxIT motif) and "leucine-x-valine-proline" (LxVP motif). In them, the x position can be occupied by different amino acids. The amino-terminal region also includes several key serine residues that can be phosphorylated by diverse kinases and dephosphorylated by calcineurin (Li et al. 2011). Besides these functional features, this region contains a small domain with transactivation function. Their DNA-binding domain (DBD),



Nuclear Factor of Activated T Cells (NFAT), Fig. 1 *Schematic representation of NFAT proteins and their functional domains*. The diagrams in (**a**) and (**b**) represent schematically the main domains of the calcineurin-dependent NFAT proteins (**a**) (NFAT1/NFATc2, NFAT2/NFATc1, NFAT3/NFATc4, and NFAT4/NFATc3) and the calcineurin-independent NFAT5 (**b**). In (**a**), the enlarged section illustrates the characteristic amino-terminal region with the calcineurin (CN)-docking sites corresponding to the amino acid motifs Pro-x-Ile-x-Ile-Thr (PxIxIT) and Leu-x-Val-Pro (LxVP) (the x position can be occupied by different amino acids), the clusters of phosphorylation sites, and the nuclear localization signal (NLS). The DNA-binding domain is indicated as DBD. The transactivation domains in the amino- and carboxy-terminal regions are indicated as TAD. (**b**) Representation of NFAT5, with the nuclear export (NES) and import (NLS) signals in its amino-terminal region, the DBD, and the long carboxy-terminal region with two glutamine-rich regions (Q). The main transactivation domain of NFAT5 is located in its carboxy-terminal region, although a smaller TAD has been described in the amino-terminal region of one of its isoforms. (**c**) Represents the main features of the DNA-binding domains (DBD) of the calcineurin-dependent NFATs and NFAT5. In the former, the residues involved in contacting with Fos/Jun and Foxp3 are not clustered in a discrete motif but located at separated positions in the primary amino acid sequence. The homodimerization domain of NFAT5 is recognizably similar to that of NF-κB proteins but different to that of the calcineurin-dependent NFATs

also referred to as Rel-homology region (RHR), is about 250 amino acids long, and besides conferring specific DNA recognition, it contains specific residues that enable these NFATs to interact with Fos and Jun proteins (Fig. 1). Most of the residues involved in contacts with Fos and Jun in the calcineurin-dependent NFATs are not conserved in NFAT5. The calcineurin-regulated NFATs contain distinct carboxy-terminal regions of varying length, which have transactivation function and are different for each NFAT (Fig. 1). In addition, several isoforms have been described for the various calcineurin-dependent NFATs. These variants differ mainly in their amino- and carboxy-terminal regions and have been shown to have specific functional properties.

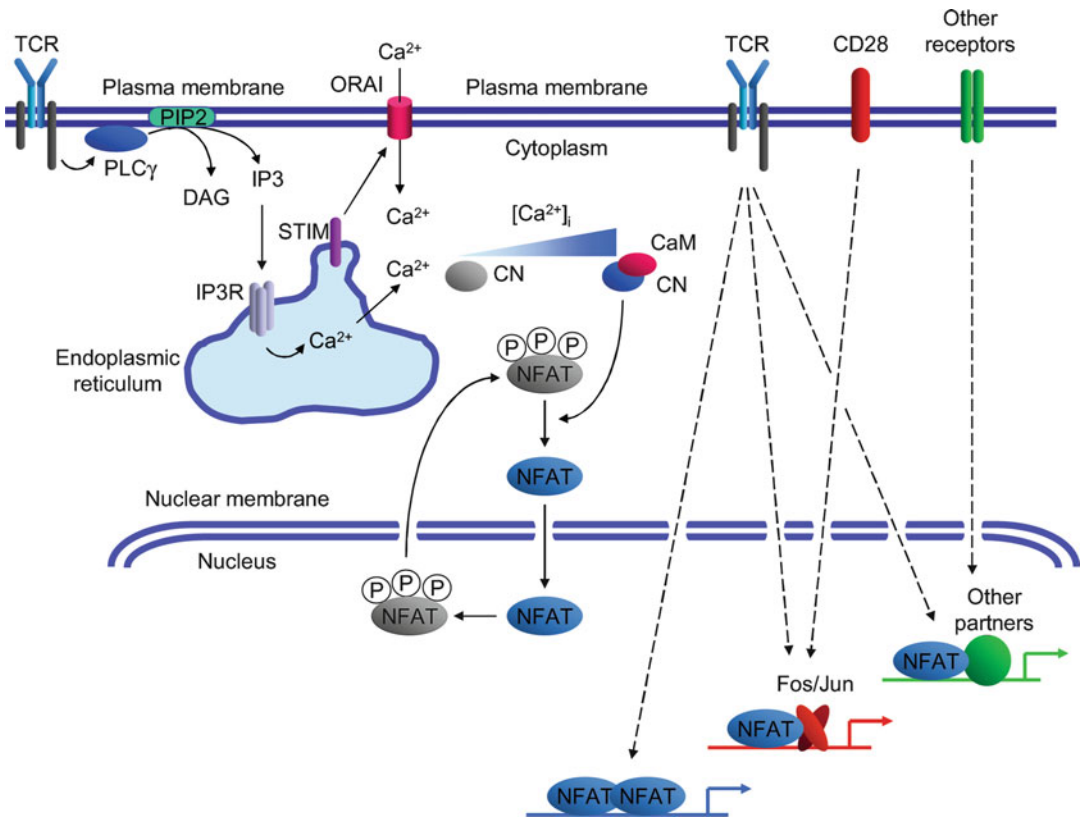
The DNA sequence recognized by the DNA-binding domain (DBD) of NFATs contains a core element "guanine-guanine-adenine-adenine-(adenine/guanine)" (GGAA(A/G)), which can be flanked by different combinations of nucleotides. One important characteristic of the

calcineurin-dependent NFATs is their ability to bind what are known as composite sites in DNA in association with other transcription factors such as homodimers of Jun/Jun, heterodimers of Fos/Jun, or Foxp3 (Macian 2005; Muller and Rao 2010). A prototypical composite site is the ARRE2 found in the enhancer of interleukin-2 (IL-2), an essential T lymphocyte growth factor. As shown for NFAT1, its binding affinity for this site is substantially enhanced by associating with the Fos/Jun heterodimer, which in turn also requires NFAT1 for efficient binding to this site. Importantly, despite that NFAT1 can make specific protein-protein contacts with Fos and Jun, it does not associate with them unless both are simultaneously bound to the appropriate DNA composite sequence and form a ternary complex NFAT-Fos/Jun-DNA. Regarding NFAT5, its DBD can bind the same type of DNA sequence as other NFATs but requires a longer core element "deoxythymidine-deoxyguanosine-deoxyguanosine-deoxyadenosine-deoxyadenosine-deoxyadenosine" (TGGAAA) (Lopez-Rodriguez et al. 1999). Therefore, sites that could be potentially bound by calcineurin-dependent NFATs may not be recognized by NFAT5.

A key functional feature shared by all the calcineurin-dependent NFATs is that their activation is strictly dependent on being dephosphorylated by the calcium- and calmodulin-activated serine/threonine phosphatase calcineurin, also known as PP2B (Fig. 2). Calcineurin is activated in response to elevations of the cytosolic concentration of calcium ion Ca^{2+}, which occurs in response to the engagement of surface receptors capable of activating phospholipase C gamma (PLCγ). Activated PLCγ hydrolyzes the plasma membrane phospholipid component phosphatidylinositol-4,5-bisphosphate (PIP2) to yield diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3). IP3 binds to a specific receptor in the endoplasmic reticulum and induces the release of stored Ca^{2+} into the cytosol, giving rise to a first wave of calcineurin activation, mediated by its association with calcium-bound calmodulin. This initial increase in the concentration of cytosolic calcium ($[Ca^{2+}]_i$) causes the activation of calcium-activated Ca^{2+} channels in the plasma membrane, such as ORAI, causing the influx of extracellular calcium and a further increase of the $[Ca^{2+}]_i$, which in turn causes the activation of a greater number of calcineurin molecules (Muller and Rao 2010). In the absence of calcium-mobilizing stimuli, NFATs are phosphorylated at multiple serines in their amino-terminal region, and in this state, they are maintained in the cytosol and prevented from translocating to the nucleus. The inhibitory phosphorylation of NFAT is maintained by diverse kinases, some of them constitutively active, such as glycogen synthase kinase 3 (GSK3), casein kinase 1 (CK1), and the dual-specificity tyrosine-phosphorylation-regulated kinase (Dyrk), and others inducible in response to diverse stimuli, such as the stress-activated kinase p38, protein kinase A (PKA), and c-Jun N-terminal kinase (JNK) (Macian et al. 2001; Muller and Rao 2010). Interestingly, JNK has been shown to have both inhibitory and stimulatory effects on different NFATs, suggesting that the positive or negative effect of specific kinases on some NFATs might be regulated by the context of other signaling pathways occurring simultaneously.

Although specific aspects of this regulation have been worked out for different NFATs, it is generally accepted that the four calcineurin-dependent NFATs are regulated by a common mechanism based on a conformational change of the amino-terminal region controlled by its phosphorylation status. Phosphorylated NFATs have their nuclear localization signal (NLS) inaccessible to the import machinery, whereas when dephosphorylated, they adopt a different conformation that exposes the NLS (Fig. 2).

When calcineurin is activated, NFATs are dephosphorylated and translocate to the nucleus, where they will bind to specific sites in regulatory regions of their target genes. Calcineurin-mediated dephosphorylation not only enables the nuclear translocation of NFATs but also enhances their DNA-binding capacity and transcriptional activity. Upon cessation of the stimulus and decrease in the $[Ca^{2+}]_i$, calcineurin is inactivated and NFAT rephosphorylated, which causes its dissociation from DNA and export out of the nucleus (Fig. 2).



Nuclear Factor of Activated T Cells (NFAT), Fig. 2 *Schematic representation of the activation cycle of the calcineurin-dependent NFAT proteins*. Phospholipase C gamma (PLCγ) activated by the T cell receptor (TCR) hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) in the plasma membrane to yield diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP3). IP3 binds to a specific receptor (IP3R) in the endoplasmic reticulum and induces the release of Ca^{2+} into the cytoplasm. Calcium mobilization can be induced by different types of surface receptors that signal through PLCγ. The ER protein STIM senses the decrease in intra-ER calcium concentration and activates the calcium channel protein ORAI in the plasma membrane, giving rise to the entry of calcium from the extracellular space and a further increase the concentration of cytosolic calcium ($[Ca^{2+}]_i$). Calcium binds to calmodulin (CaM), which in turns activates calcineurin (CN). Calcineurin dephosphorylates the amino-terminal region of NFAT, which undergoes a conformational change that exposes its nuclear localization signal. The nuclear import/export cycle of the calcineurin-dependent NFATs is controlled by the balance between active calcineurin and opposing kinases that phosphorylate the amino-terminal region of NFAT. NFAT can bind DNA alone or in association with other transcriptional partners, such as Fos/Jun, activated by the TCR or other signaling pathways. These functional associations enable NFATs to regulate different transcriptional programs in diverse contexts

NFAT5/TonEBP. This protein is similar to other NFATs only at the level of its DBD, which shows about 45 % homology with those of the calcineurin-dependent NFATs (Aramburu et al. 2006) (Fig. 1). Three NFAT5 isoforms differing in the beginning portion of their amino-terminal regions have been described. NFAT5 binds DNA elements very similar to those recognized by the calcineurin-dependent NFATs, and indeed, its DNA recognition motif "arginine-alanine-arginine-tyrosine-leucine-threonine-glutamic acid-glycine" (RARYLTEG) is highly similar to that of the other NFATs, "arginine-alanine-histidine-tyrosine-glutamic acid-threonine-glutamic acid-glycine" (RAHYETEG). On the other hand, while calcineurin-dependent NFATs can bind DNA efficiently as monomers or dimers depending on the DNA site, NFAT5 is a constitutive homodimer

and needs to be dimeric to bind DNA (Lopez-Rodriguez et al. 2001). It also lacks most of the Fos/Jun-interaction residues and is unable to associate with Fos/Jun at composite sites such as the ARRE2 (Lopez-Rodriguez et al. 1999). Notably, the dimeric structure of NFAT5 resembles that of NF-κB proteins, which constitute a group of transcription factors related to NFATs by virtue of the similarity of their DBD, generically known as Rel-like DBD. In this regard, the dimerization interface of NFAT5 is recognizably similar to that of NF-κB proteins (Lopez-Rodriguez et al. 2001; Aramburu et al. 2006).

The two main distinguishing features of NFAT5 that set it apart from the calcineurin-dependent NFATs are its ability to be activated by hyperosmotic stress and its lack of a calcineurin-regulated module with calcineurin-docking sites and regulatory phosphoserines to control its nucleocytoplasmic localization (Aramburu et al. 2006). Because of these characteristics, some researchers consider NFAT5 not to be a genuine NFAT and prefer to refer to it as TonEBP to indicate its osmotic stress response function. Nonetheless, although NFAT5 has been more extensively characterized as a regulator of osmostress responses, it also has osmostress-independent functions.

In response to osmotic stress, such as when cells are exposed to fluids with elevated sodium concentration, NFAT5 activates the expression of genes whose products encode for enzymes and transporters that allow cells to accumulate by synthesis or uptake from the extracellular medium, substances known as compatible osmolytes. These osmolytes, generally derived from sugars or amino acids, prevent the excessive buildup of sodium ions, and hence ionic strength, that occurs when cells are exposed to hypertonic solutions. Since elevation of the intracellular ionic strength above physiologic levels can be harmful for numerous cellular processes, NFAT5 plays an essential role in allowing cells to survive and maintain proliferative capacity for extended periods under hyperosmotic stress. This function is clearly illustrated by the severe renal phenotype of NFAT5-deficient mice. As cells in the renal medulla are naturally exposed to elevated hypertonicity levels, their survival is dependent on NFAT5, and lack of this factor causes atrophy of the renal medulla and pathological alterations of the morphology and function of the kidney (Aramburu et al. 2006). Besides the kidney, NFAT5 is expressed in essentially all types of mammalian cells, and its osmoprotective function has been shown in cell types as diverse as neurons, nucleus pulposus cells, cardiomyocytes, skeletal muscle, fibroblasts, macrophages, and lymphocytes.

The fact that NFAT5 is expressed in many cell types led researchers to investigate whether NFAT5 could also have osmostress-independent functions. Although this area is still less explored than its function under osmostress, already a number of osmotic stress-independent roles have been identified for NFAT5: it regulates migration in response to integrin signaling in certain carcinoma cell lines, myoblast differentiation, resistance of cardiomyocytes to some chemotherapeutic drugs, development of the embryonic heart, and macrophage activation and inflammatory responses to pathogen-derived products. It is expected that future research will uncover novel functions for this factor.

NFAT Proteins in Immunity

Calcineurin-Dependent NFAT Proteins. Through almost 25 years of recorded NFAT history, the calcineurin-dependent NFATs have been shown to be major regulators of gene expression in immune cells in response to a wide variety of calcium mobilization-coupled receptors. The molecular cloning of individual NFATs combined with gene-targeting strategies in mice has yielded a wealth of information about their functions in different cell types and immune processes. The calcineurin-dependent NFATs regulate the proliferative capacity of T cells, sensitivity to apoptosis inducers, their ability to differentiate towards specialized effector and regulatory phenotypes, and induction of anergy, this latter characterized by the inability of T cell receptor-stimulated lymphocytes to respond to a new restimulation of their T cell receptor (TCR).

This they do by regulating the expression of genes whose products work intracellularly, as well as others encoding for surface receptors and secreted cytokines (Hogan et al. 2003; Macian 2005; Muller and Rao 2010; Hermann-Kleiter and Baier 2010).

The calcineurin-dependent NFATs have been shown to regulate functions of CD4 and CD8 T cells, as well as other types of immune cells, including B lymphocytes, NK cells, macrophages, dendritic cells, and mast cells (Rao et al. 1997). Besides the TCR, different receptors in diverse immune cells can activate NFATs: for instance, the B cell receptor and CD40 in B lymphocytes, Fc receptors in macrophages and NK cells, activating receptors for the major histocompatibility complex (MHC)-class I in NK cells, immunoglobulin E (IgE) receptor in mastocytes, or the C-type lectin carbohydrate receptor Dectin-1 in macrophages and dendritic cells (Muller and Rao 2010).

NFAT functions have been more extensively characterized in $CD4^+$ T lymphocytes, and this section highlights some of the key features of these factors. T cells express three calcineurin-dependent NFATs: NFAT1/NFATc2, NFAT4/NFATc3, and NFAT2/NFATc1. These NFATs can have partially overlapping or redundant functions but also specific ones. For instance, production of IL-2 or tumor necrosis factor-alpha (TNF-α) can be induced by any of the three NFATs, whereas other functions are more selectively dependent on one or another NFAT. This is the case of T cell specialization towards IL-4-producing T helper type 2 (Th2), which is facilitated in the absence of NFAT1 but less effective in lymphocytes lacking NFAT2. The ability of NFAT to cooperate with specific transcription factor partners allows that different combination of signals transduced by surface receptors will give rise to distinct profiles of expressed NFAT-regulated genes. Relevant variables in how NFATs shape particular gene expression profiles are the duration and magnitude of their activation resulting from varying intensities of receptor stimulation, the relative abundance of each NFAT and its respective isoforms, and their temporal overlapping with windows in which other transcription factors such as Fos/Jun or Foxp3 are activated in response to other signals. As an illustrative example of different outcomes regulated by a single NFAT, NFAT1 can promote T cell anergy if cells do not receive appropriate costimulatory signals when activated by their TCR. In this case, NFAT1 induces a particular anergy-associated gene expression program. On the other hand, NFAT1 will enhance T cell activation towards effector phenotypes when T cells receive simultaneous signaling from the TCR in concert with costimulatory receptors such as CD28. Finally, NFAT1 can promote differentiation of inducible T regulatory cells (iTreg) by cooperating with Foxp3 (Muller and Rao 2010).

In summary, the expression of several calcineurin-dependent NFATs in T cells, together with the different functional properties of each NFAT and their ability to interact functionally with other transcriptional regulators, endows T cells with a versatile module to translate signals received from calcineurin-activating receptors in combination with other signals to induce a complex variety of gene expression patterns.

NFAT5/TonEBP. The function of NFAT5 in T cells is much less explored than that of the calcineurin-dependent NFATs. As known for other cell types, NFAT5 plays an important osmoregulatory role in T cells, and this function is required for survival and cell-cycle progression of T cells under osmotic stress in vitro and in vivo. In this regard, NFAT5-deficient mice suffer T cell immunodeficiency, which is in part due to inefficient adaptation to the chronic hypernatremia observed in these mice (Berga-Bolaños et al. 2010). Notably, NFAT5 activated by osmotic stress does not only induce gene products with a direct osmoprotective function but can promote the expression of cytokines, such as TNF-α or lymphotoxin-β, and is a potent transcriptional inducer of CD24, a surface glycoprotein involved in homeostatic cell survival and proliferation, in mature T lymphocytes (Berga-Bolaños et al. 2010).

These observations have suggested that NFAT5 may not just regulate T cell survival under osmostress but perhaps other T cell functions in specific stress contexts.

Independently of its osmoresponsive function, NFAT5 has been recently found to respond to microbial products via Toll-like receptors in macrophages and regulate the expression of inducible nitric oxide synthase (iNOS) and different inflammatory cytokines (Buxadé et al. 2012). This novel function of NFAT5 in anti-pathogen responses reveals a relevant function for this factor in the immune system beyond its previously known osmoregulatory role.

Functions of NFAT Proteins Beyond the Immune System

Essentially, all nucleated cell types express calcineurin-dependent NFATs and NFAT5. The former have been shown to regulate the development of the embryonic cardiovascular system, cardiac function in the adult as well as pathologic cardiac hypertrophy, angiogenesis and activity of endothelial cells, skeletal and smooth muscle cell function, neuronal function, and even glucose and insulin metabolism. Another important aspect of calcineurin-dependent NFATs is their involvement in cancer, both of hematopoietic origin and other types, by virtue of their ability to influence, positively or negatively, the expression of cell-cycle regulatory proteins such as cyclin A and cyclin-dependent kinase 4 (CDK4), apoptotic inducers such as Fas ligand, processes important for metastasis (cellular chemotaxis, invasion and migration capacity of tumor cells), and support of tumor growth via angiogenesis (Muller and Rao 2010; Gachet and Ghysdael 2009; Mancini and Toker 2009). The wide range of processes in multiple cell types that are sensitive to the signaling-transcription axis formed by calcineurin and NFATs make this pathway attractive for pharmacological intervention in different scenarios but also pose the problem of achieving selectivity.

Pharmacological Relevance of NFAT Proteins

Even before NFATs were identified and their functional features elucidated, two microbial products, cyclosporin A produced by the fungus *Tolypocladium inflatum* and FK506 (tacrolimus) produced by the bacterium *Streptomyces tsukubaensis*, were revolutionizing the field of transplantation due to their potent immunosuppressive effect. Both compounds were found to inhibit the same intracellular target, calcineurin. They do this upon binding to intracellular proteins generically known as immunophilins. While neither compound can bind to calcineurin, the respective complexes formed by cyclosporin A associated with cyclophilin, and FK506 associated with FK506-binding protein 12 (FKBP12), are able to bind with very high affinity to the A subunit of calcineurin near its active site (Li et al. 2011). Of note, another bacterial compound with immunosuppressive properties, rapamycin (also known as sirolimus), produced by the bacterium *Streptomyces hygroscopicus*, also works by forming a complex with FKBP12, although the target of this complex is not calcineurin but the kinase mTOR, a central regulator of cell growth and biosynthesis. Since the finding that calcineurin was essential for the function of NFATs, it became clear that one key immunosuppressive mechanism of cyclosporin A and FK506 was the inhibition of these factors.

Different approaches in cells or mouse models have shown that suppression or overactivation of NFAT function, either directly or by interfering with calcineurin and NFAT-regulatory kinases, can affect numerous processes from immune responses to cardiovascular function to tumor progression. Thus, the notion of pharmacologic manipulation of NFATs for therapeutic intervention would be attractive in different scenarios. Potential approaches might fall into four main categories: (a) targeting signaling pathways that control the activity of calcineurin and NFAT-regulatory kinases or pathways impinging on the activation of NFAT transcriptional

partners; (b) interfering with protein-protein interactions between NFATs and regulators such as calcineurin or particular transcriptional partners, such as AP-1 or Foxp3; (c) altering the expression level of endogenous NFATs and their regulators by, for instance, using RNA interference or other approaches that could increase or decrease their stability and degradation; and (d) targeting the expression or activity of NFAT-induced gene products. At present, the calcineurin inhibitors cyclosporin A and FK506 are the only drugs targeting the direct activation of NFAT that are used in the clinic, but it can be expected that future studies might spur the development of therapeutic approaches based on these factors. In this regard, one avenue towards developing NFAT-selective inhibitors was opened by the finding that calcineurin needs to dock at specific sites (the PxIxIT and LxVP motifs) on NFATs in order to dephosphorylate them (Li et al. 2011). Experiments showed that intracellular expression of short peptides with PxIxIT or LxVP-like sequences could inhibit the activity of NFATs in live cells. A further development came with the identification of small molecule chemicals that bound to calcineurin and impaired its interaction with the PxIxIT site of NFATs (Roehrl et al. 2004). These cell-permeant compounds were capable of inhibiting the activation of NFATs by calcineurin and induction of NFAT-dependent cytokine genes without impairing the phosphatase activity of calcineurin. Although further research will be needed to elucidate whether these compound, or others using a similar mechanism of action, can be made into effective NFAT inhibitors in animal models and finally translated to humans, these findings provide a stimulating example of the potential for the development of novel types of NFAT modulators.

Cross-References

- ▶ Cytotoxic T Lymphocytes
- ▶ Dendritic Cells in Atherosclerosis
- ▶ Immunodeficiency in Autoimmune Diseases
- ▶ Immunosuppression in Clinical Liver Transplantation
- ▶ NF-κB
- ▶ PI3K
- ▶ T Cell Memory
- ▶ Tregs in the Liver

References

Aramburu J, Drews-Elger K, Estrada-Gelonch A, Minguillon J, Morancho B, Santiago V, Lopez-Rodriguez C. Regulation of the hypertonic stress response and other cellular functions by the Rel-like transcription factor NFAT5. Biochem Pharmacol. 2006;72:1597–604.

Berga-Bolaños R, Drews-Elger K, Aramburu J, Lopez-Rodriguez C. NFAT5 regulates T lymphocyte homeostasis and CD24-dependent T cell expansion under pathologic hypernatremia. J Immunol. 2010;185:6624–35.

Buxadé M, Lunazzi G, Minguillón J, Iborra S, Berga-Bolaños R, del Val M, Aramburu J, López-Rodríguez C. Gene expression induced by Toll-like receptors in macrophages requires the transcription factor NFAT5. J Exp Med. 2012;209:379–93.

Gachet S, Ghysdael J. Calcineurin/NFAT signaling in lymphoid malignancies. Gen Physiol Biophys. 2009;28:F47–54.

Graef IA, Gastier JM, Franke U, Crabtree GR. Evolutionary relationships among Rel domains indicate functional diversification by recombination. Proc Natl Acad Sci USA. 2001;98:5740–5.

Hermann-Kleiter N, Baier G. NFAT pulls the strings during CD4+ T helper cell effector functions. Blood. 2010;115:2989–97.

Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev. 2003;17:2205–32.

Jain J, McCaffrey PG, Valge-Archer VE, Rao A. Nuclear factor of activated T cells contains Fos and Jun. Nature. 1992;356:801–4.

Li H, Rao A, Hogan PG. Interaction of calcineurin with substrates and targeting proteins. Trends Cell Biol. 2011;21:91–103.

Lopez-Rodriguez C, Aramburu J, Rakeman AS, Rao A. NFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun. Proc Natl Acad Sci USA. 1999;96:7214–9.

Lopez-Rodriguez C, Aramburu J, Jin L, Rakeman AS, Michino M, Rao A. Bridging the NFAT and NF-kappaB families: NFAT5 dimerization regulates cytokine gene transcription in response to osmotic stress. Immunity. 2001;15:47–58.

Macian F. NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol. 2005;5:472–84.

Macian F, Lopez-Rodriguez C, Rao A. Partners in transcription: NFAT and AP-1. Oncogene. 2001;20: 2476–89.

Mancini M, Toker A. NFAT proteins: emerging roles in cancer progression. Nat Rev Cancer. 2009;9:810–20.

McCaffrey PG, Luo C, Kerppola TK, Jain J, Badalian TM, Ho AM, Burgeon E, Lane WS, Lambert JN, Curran T, Verdine GL, Rao A, Hogan PG. Isolation of the cyclosporin-sensitive T cell transcription factor NFATp. Science. 1993;262:750–4.

Miyakawa H, Woo SK, Dahl SC, Handler JS, Kwon HM. Tonicity-responsive enhancer binding protein, a rel-like protein that stimulates transcription in response to hypertonicity. Proc Natl Acad Sci USA. 1999;96:2538–42.

Muller MR, Rao A. NFAT, immunity and cancer: a transcription factor comes of age. Nat Rev Immunol. 2010;10:645–56.

Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. Annu Rev Immunol. 1997;15:707–47.

Roehrl MH, Kang S, Aramburu J, Wagner G, Rao A, Hogan PG. Selective inhibition of calcineurin-NFAT signaling by blocking protein-protein interaction with small organic molecules. Proc Natl Acad Sci USA. 2004;101:7554–9.

Shaw JP, Utz PJ, Durand DB, Toole JJ, Emmel EA, Crabtree GR. Identification of a putative regulator of early T cell activation genes. Science. 1988;241:202–5.

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Paraneoplastic Neurological Syndromes, Overview

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Definition

Paraneoplastic neurological syndromes (PNS) are a group of disorders resulting from damage to the nervous system in the setting of cancer, but remote from the site of cancer, and not related to metastasis, infection, or metabolic derangements otherwise associated with cancer. They result from direct damage to neural tissue through an immune-mediated mechanism itself triggered either by an immunologic disturbance caused by the tumor or by tumors that express neuronal proteins. PNS may affect either the central or peripheral nervous system or both, and they may be isolated to a single neuronal cell type or site or affect more diffuse involvement of the nervous system. Antineuronal antibodies, useful as diagnostic markers of PNS and the underlying cancer, are often associated with these immune responses, some being strongly associated with specific tumor types, some less so (Graus et al. 2004). Several recognizable clinical syndromes have been identified (Table 1) and will be the focus of discussion in this entry.

Clinical Features

Symptom onset is usually acute to subacute, over weeks to months, producing severe progressive disability followed by stabilization. While PNS may present in a known cancer patient, most commonly it is the presenting symptom in a previously healthy person. Consequently, as the neurologist will likely be the first physician to encounter the patient given the neurologic nature of these syndromes, it is important for her/him to be familiar with these syndromes, recognize them as possibly paraneoplastic, and initiate a search for an underlying neoplasm. Most PNS are neurologically debilitating and, given an underlying pathology of immune-mediated neuronal loss, predominately irreversible.

Paraneoplastic syndromes are often associated with evidence of inflammation in the nervous system including pleocytosis (30–40 cells/ml), elevated protein levels (50–100 mg/dL), and positive oligoclonal bands in the cerebrospinal fluid (Darnell and Posner 2003). Inflammatory infiltration of tumor tissue in PNS suggests a similar immune attack against the tumor itself. Imaging studies of the nervous system are usually normal, although there may be abnormalities on magnetic resonance imaging (MRI), such as hyperintense

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Paraneoplastic Neurological Syndromes, Overview, Table 1 Paraneoplastic syndromes of the nervous system

Central nervous system
Multifocal encephalomyelitis
Limbic encephalitis
Anti-NMDA receptor encephalitis
Cerebellar degeneration
Opsoclonus-myoclonus
Optic neuritis
Retinal degeneration
Brainstem encephalitis
Stiff person syndrome
Motor neuron disease and motor neuronopathy
Necrotizing myelopathy
Chorea
Parkinsonism
Tremor
Peripheral nervous system
Sensory neuronopathy
Motor neuropathy
Acute sensorimotor neuropathy (Guillain-Barre, Plexitis)
Chronic sensorimotor neuropathy
Neuropathy associated with malignant monoclonal gammopathies
Vasculitis of nerve and muscle
neuromyotonia
Chronic gastrointestinal pseudo-obstruction/autonomic neuropathy
Lambert-Eaton myasthenic syndrome
Myasthenia gravis
Inflammatory myopathy
Acute necrotizing myopathy

T2/fluid-attenuated inversion recovery (FLAIR) signal and contrast enhancement of the mesial temporal lobes in limbic encephalitis (Lawn et al. 2003) (see Pathology below).

Another unifying characteristic of PNS is the discovery of antibodies against neural tissue in serum and cerebrospinal fluid. Many of these antibodies have been well characterized through the use of antiserum samples from patients with PNS to screen complementary DNA libraries to determine the target antigens (Darnell 2004, Musunuru and Darnell 2001). These target antigens are termed onconeural antigens and are expressed by both the tumor cells and neuronal tissue. Discovery of these antibodies aids in diagnosis, especially when an occult malignancy is undetectable. Many of these antibodies, some named after the surname of the index patient, are specific to one or a group of clinical syndromes as well as tumor type, suggesting the potential site of an underlying cancer (anti-Hu antibody suggests small-cell lung cancer, anti-Yo suggests breast or ovarian cancer) (Table 2). Although the presence of paraneoplastic antibodies in the appropriate clinical setting is virtually diagnostic, the yield can be low, less than 1 % in a large study from the Mayo Clinic (Pottock et al. 2004). Furthermore, the presence of antibodies does not confer disease, as some paraneoplastic antibodies are present in cancer patients without a PNS, usually in low levels, and in <1 % of healthy individuals (Raspotnig et al. 2011).

Epidemiology

PNS are rare, estimated to occur in 0.01 % of cancer patients (Darnell and Posner 2003), but certain malignancies are more commonly associated with PNS. Fewer than 1 % of breast or ovarian cancer patients develop PNS, approximately 1–3 % of patients with small-cell lung cancer develop Lambert-Eaton myasthenic syndrome (Suete et al. 2004), 40 % of thymoma patients develop myasthenia gravis (Vernino and Lennon 2004), and 10 % of monoclonal gammopathy patients develop peripheral neuropathy (Latov 1995).

Other malignancies commonly associated with PNS include Hodgkin's and non-Hodgkin's lymphoma, testicular cancer, and neuroblastoma. However, virtually any neoplasm may be associated with a paraneoplastic syndrome (Bataller and Dalmau 2003).

Diagnosis

Over the years, certain clinical presentations have been found to represent classic paraneoplastic syndromes and to be associated with certain malignancies and well-characterized onconeural antibodies. There is, however, significant variability. Not all patients with PNS have paraneoplastic antibodies and not all patients with paraneoplastic antibodies have a PNS.

Paraneoplastic Neurological Syndromes, Overview, Table 2 Antibodies associated with paraneoplastic neurologic disorders

Antibody	Associated cancer	Syndrome
Antibodies that are markers of paraneoplasia		
Anti-Hu	SCLC, other	Encephalomyelitis, sensory neuronopathy
Anti-Yo	Gynecological, breast	Cerebellar degeneration
Anti-Ri	Breast, gynecological,	SCLC cerebellar ataxia, opsoclonus, brainstem encephalitis
Anti-Tr	Hodgkin's lymphoma	Cerebellar degeneration
Anti-CV2/CRMP5	SCLC, thymoma, other	Encephalomyelitis, striatal encephalitis (chorea), cerebellar degeneration, uveitis, peripheral neuropathy
[a]Anti-Ma proteins	Testicular germ cell tumors and other neoplasms	Limbic, diencephalic (hypothalamic), and upper brainstem encephalitis; rarely cerebellar degeneration
Anti-amphiphysin	Breast, SCLC	Stiff man syndrome, encephalomyelitis
Anti-NMDAR	Teratoma of the ovary or mediastinum	Encephalitis with predominant psychiatric symptoms, autonomic dysfunction, dyskinesias, hypoventilation.
		Infrequently presents as classical limbic encephalitis
Antibodies that are not markers of paraneoplasia		
(Associated with the indicated neurologic syndromes whether they are paraneoplastic or not)		
Anti-VGKC	Thymoma, SCLC	Neuromyotonia, limbic encephalitis, Morvan's syndrome
Anti-VGCC	SCLC	LEMS, cerebellar degeneration
Anti-AChR	Thymoma	Myasthenia gravis
(*muscle*)		
Anti-AChR	SCLC and other	Autonomic neuropathy
(*neuronal*)		

[a]Antibodies limited to Ma2 (also called anti-Ta antibodies) usually associate with limbic and brainstem encephalitis and germ cell tumors. Antibodies directed at Ma1 and Ma2 usually associate with brainstem encephalitis, cerebellar degeneration, and several types of cancer (lung, breast, ovary, among others). *Antibodies in italics* denote that are directed against cell membrane antigens. The other antibodies are directed against intracellular antigens. *NMDAR* *N*-methyl-d-aspartate receptor, *VGKC* voltage-gated potassium channels, *VGCC* voltage-gated calcium channels, *LEMS* Lambert-Eaton myasthenic syndrome, *AChR* acetylcholine receptor (From Paraneoplastic Syndromes of the Nervous System. Myrna R. Rosenfeld MD, PhD, and Josep Dalmau, MD, PhD. Originally published in *Current Clinical Oncology*: *Cancer Neurology in Clinical Practice*, Edited by: D. Schiff, S. Kesari, and P. Y. Wen © Humana Press, USA part of Springer Science+Business Media. 2010. With permission)

Furthermore, there are patients in whom no tumor is ever detected despite presenting with classic PNS and high titers of a well-characterized onconeural antibody (Greenlee 2004).

To address the diagnostic difficulty, a panel of neurologists developed guidelines to provide more rigorous diagnostic criteria for PNS (Graus et al. 2004). The panel began by defining a list of "classical" syndromes, which included encephalomyelitis, limbic encephalitis, subacute cerebellar degeneration, opsoclonus-myoclonus, subacute sensory neuropathy, chronic gastrointestinal pseudo-obstruction, Lambert-Eaton myasthenic syndrome, and dermatomyositis. Myasthenia gravis and paraproteinemic neuropathies were excluded from this list, as historically they are discussed in other clinical forums. The panel then defined a list of "well-characterized" paraneoplastic antibodies, based on their association with neurological syndromes, the number of case reports, their frequency in patients without cancer, and their identification among different studies. These include anti-Hu, anti-Yo, anti-CV2, anti-Ri, anti-Ma2, and anti-amphiphysin. Diagnostic criteria were then created to define a given neurological syndrome as "definite" or "possible" PNS (Table 3).

As noted, PNS usually presents in patients with no known cancer history. In 80 %, cancer will be diagnosed within months to years (Honnorat and Antoine 2007). If the symptoms represent a classic PNS or if PNS is suspected,

Paraneoplastic Neurological Syndromes, Overview, Table 3 Diagnostic criteria for paraneoplastic neurological syndromes

Definite PNS
1. A classical syndrome and cancer that develops within 5 years of the diagnosis of the neurological disorder
2. A nonclassical syndrome that resolves or significantly improves after cancer treatment without concomitant immunotherapy provided that the syndrome is not susceptible to spontaneous remission
3. A nonclassical syndrome with onconeural antibodies (well characterized or not) and cancer that develops within 5 years of the diagnosis of the neurological disorder
4. A neurological syndrome (classic or not) with well-characterized onconeural antibodies (anti-Hu, Yo, CV2, Ri, Ma2, amphiphysin) and no cancer
Possible PNS
1. A classical syndrome, no onconeural antibodies, no cancer but a high risk to have an underlying tumor
2. A neurological syndrome (classic or not) with partially characterized onconeural antibodies and no cancer
3. A nonclassical syndrome, no onconeural antibodies, and cancer present within 2 years of diagnosis

(Graus et al. 2004, with permission)

MRI, neurophysiologic studies, cerebrospinal fluid analysis, and evaluation for serum paraneoplastic antibodies are warranted. If onconeural antibodies are present or if clinical suspicion is high, a search for underlying cancer is undertaken with both full body CT and ^{18}F fluoro-2-deoxy-glucose (FDG)-positron emission tomography (PET) scanning to increase sensitivity of tumor diagnosis (Younes-Mhenni et al. 2004).

If cancer is not detected initially, continued surveillance at regular intervals is necessary (Graus et al. 2001; Raspotnig et al. 2011; Titulaer et al. 2011). If the cancer detected is not one commonly associated with a classical syndrome, further evaluation is recommended to identify a second, more typical malignancy, unless it can be demonstrated that the primary tumor expresses the onconeural antigen in question (Graus et al. 2001, 2004).

Pathophysiology

Currently, autoimmunity is postulated to underlie the pathophysiology of PNS. In PNS patients, tumor cells express antigens normally found only in the nervous system, an immunologically privileged site (Darnell and Posner 2006). The onconeural antigen is identified by the immune system as foreign; the body mounts an immune attack, produces an antitumor immune response, and suppresses tumor growth, indeed accounting for the difficulty at presentation in identifying the underlying cancer (Graus et al. 2001). An antitumor immune response is commonly seen without an autoimmune component, given the presence of paraneoplastic antibodies in non-PNS patients with cancer, and may portend compete response to therapy and improved survival (Darnell and DeAngelis 1993).

In a subset of PNS patients, the immune system recognizes onconeural antigens in normal nervous tissue and mounts an immune attack against that normal tissue. While paraneoplastic antibodies may be present in non-PNS patients with cancer, titers are usually considerably higher in PNS patients (Roberts and Darnell 2004). High cerebrospinal fluid (CSF) paraneoplastic antibody titers in PNS affecting the brain indicate local synthesis within the central nervous system, from B cells that crossed the blood–brain barrier.

The function of paraneoplastic antigens in PNS remains unclear but may relate to the relative roles of humoral versus cellular immunity. Certain antibodies have a clear role in the clinical syndrome, such as voltage-gated calcium channel antibodies (VGCC-Ab) in Lambert-Eaton myasthenic syndrome (LEMS) and acetylcholine receptor antibodies (AchR-Ab) in MG, evidenced by the development of the characteristic electrophysiologic abnormalities in mice following passive transfer of purified immunoglobulin G (IgG) (Kim 1986). Similar mechanisms seem to occur in PNS that affect the nervous system outside the blood–brain barrier when the target antigens are cell surface receptors. However, many onconeural antigens are expressed intracellularly, within the blood–brain barrier, and passive transfer of these antigens into experimental animals does not produce disease or cause injury to cultured neurons in vitro (Tanaka et al. 2004). In these instances, a cellular component to the immune attack is believed to be responsible. Clear evidence of such is demonstrated in patients with paraneoplastic

cerebellar degeneration where circulating, antigen-specific, cytotoxic T lymphocytes (CTL) are present in the cerebrospinal fluid (Roberts and Darnell 2004), suggesting, in certain PNS, that while paraneoplastic antibodies may play a crucial role in diagnosis, they have little role in disease pathogenesis.

The number of known paraneoplastic antibodies has been steadily growing since the mid-1980s, suggesting that further antibodies are yet to be discovered (Table 3). Consequently, suspected PNS patients in whom no known antibody is detected may, on further testing, have a previously unrecognized antibody. Therefore, a negative antibody assay does not rule out PNS or the presence of an underlying neoplasm.

Pathology

Histologically, PNS, as a group, demonstrate neuronal loss, gliosis, microglial proliferation, and perivascular lymphocytic inflammatory infiltrates, usually T cells, but occasionally, plasma cells. In limbic encephalitis, neuronal loss and inflammation predominantly affects the medial temporal and inferior frontal lobes, the insular cortex, and the cingulate gyrus. The medulla is the primary focus of involvement in brainstem encephalitis, whereas the Purkinje cells undergo severe loss in paraneoplastic cerebellar degeneration. In the opsoclonus-myoclonus-ataxia syndrome, no specific pathology is described but Purkinje cells will be lost in 50 %. Dorsal root ganglia demonstrate inflammation and cell loss in paraneoplastic sensory neuronopathy. Electron microscopy plays no role in PNS diagnosis but may be useful in demonstrating microorganisms which can cause infectious encephalitis that mimics the histology of PNS (Fuller et al. 2010).

References

Bataller L, Dalmau J. Paraneoplastic neurologic syndromes: approaches to diagnosis and treatment. Sem in Neurol. 2003;23(2):215–24.

Darnell RB. Paraneoplastic neurologic disorders. Arch Neurol. 2004;61:30–2.

Darnell RB, DeAngelis LM. Regression of small-cell lung carcinoma in patients with paraneoplastic neuronal antibodies. Lancet. 1993;341:21–2.

Darnell RB, Posner JB. Paraneoplastic syndromes involving the nervous system. N Eng J Med. 2003;349:1543–54.

Darnell RB, Posner JB. Paraneoplastic syndromes affecting the nervous system. Semin Oncol. 2006;33:270–98.

Fuller CE, Narendra S. Paraneoplastic disorders of the central nervous system. In: Adesina AM et al., editors. Atlas of pediatric brain tumors. doi:10.1007/978-1-4419-1062-2_34, © Springer Science + Business Media, LLC 2010.

Graus F, Delattre JY, Antoine JC, et al. Recommended diagnostic criteria for paraneoplastic neurological syndromes. J Neurol Neurosurg Psychiatry. 2004;75:1135–40.

Graus F, Florence KG, Reñé R, et al. Anti-Hu associated paraneoplastic encephalomyelitis: analysis of 200 patients. Brain. 2001;124:1138–48.

Greenlee JE. Recommended diagnostic criteria for paraneoplastic neurological syndromes: editorial commentary. J Neurol Neurosurg Psychiatry. 2004;75:1090.

Honnorat J, Antoine JC. Paraneoplastic neurological syndromes. Orphanet J Rare Dis. 2007;2:22.

Kim YI. Passively transferred Lambert-Eaton syndrome in mice receiving purified IgG. Muscle Nerve. 1986;9:523–30.

Latov N. Pathogenesis and therapy of neuropathies associated with monoclonal gammopathies. Ann Neurol. 1995;37(S1):S32–42.

Lawn ND, Westmoreland BF, Kiely MJ, et al. Clinical, magnetic resonance imaging, and electroencephalographic findings in paraneoplastic limbic encephalitis. Mayo Clin Proc. 2003;78:1363–8.

Musunuru K, Darnell RB. Paraneoplastic neurologic disease antigens. Annu Rev Neurosci. 2001;24:239–62.

Pottock SJ, Kryzer TJ, Lennon VA. Paraneoplastic antibodies coexist and predict cancer, not neurological syndrome. Ann Neurol. 2004;56:715–9.

Raspotnig M, Vedeler CA, Storstein A. Onconeural antibodies in patients with neurological symptoms: detection and clinical significance. Acta Neurol Scand. 2011;124(Suppl. 191):83–8.

Roberts WK, Darnell RB. Neuroimmunology of the paraneoplastic neurological degenerations. Curr Opin Immunol. 2004;16:616–22.

Suete T, Leffers P, Ten Velde GP, et al. Neurologic disorders in 432 consecutive patients with small cell lung carcinoma. Cancer. 2004;100:801–6.

Tanaka K, Ding X, Tanaka M. Effects of antineuronal antibodies from patients with paraneoplastic neurological syndrome on primary cultured neurons. J Neurol Sci. 2004;217:25–30.

Titulaer MJ, Soffietti R, Dalmau J, et al. Screening for tumours in paraneoplastic syndromes: report of an EFNS Task Force. Eur J Neurol. 2011;18:19–27.

Vernino S, Lennon VA. Autoantibody profiles and neurological correlations of thymoma. Clin Cancer Res. 2004;10:7270–5.
Younes-Mhenni S, Janie MF, Cinotti L, et al. FDG-PET improves tumor detection in patients with paraneoplastic neurological syndromes. Brain. 2004;127:2331–8.

PBC Genetics

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Synonyms

Chronic nonsuppurative destructive cholangitis; Primary biliary cirrhosis

Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by progressive destruction of small/medium intrahepatic bile ducts which leads to cirrhosis and finally to liver transplantation or death (Invernizzi et al. 2010). PBC is a rare condition being present in about 1 of 1,000 women over the age of 40, and ursodeoxycholic acid is the only approved therapy. Liver transplantation is also a possible therapeutic option in patients with end-stage liver disease. The presence of specific serum and cell-mediated responses against defined self-antigens, the increased risk of developing PBC or another autoimmune disorder among family members, and a striking female predominance (female to male ratio up to 10:1) indicated the autoimmune pathogenesis of PBC (Invernizzi et al. 2008). The current hypothesis on its etiopathogenesis implies that the disease results from a genetic predisposition that is permissive for an unknown environmental factor (Invernizzi et al. 2010).

The Background Evidence for Genetic Predisposition in PBC

In the past, a scenario named "familial PBC" has been proposed based on a number of studies reporting an increased risk of developing PBC within family members of affected individuals (Hirschfield and Invernizzi 2011). The main part of these studies as well as population-based epidemiological reports were performed in Great Britain, where the prevalence rates of familial PBC was reported to be 6.4 % (Jones et al. 1999). A number of studies from North America, Europe, and Japan reported a similar figure ranging between 3.8 % and 9.0 %. Also the sibling relative risk, another estimate of the familial prevalence of PBC, was found to be increased (Jones et al. 1999). Finally, a recent large-scale US study showed an increased risk of disease (odds ratio at 10.7) in first-degree relative with PBC (Gershwin et al. 2005). Of course, these findings might be explained by some shared environmental factors by family members. Also the coexistence with other autoimmune diseases in more than one third of patients with PBC strongly suggest a role for genetic factor in this disease (Gershwin et al. 2005). Again, by evaluating eight monozygotic and eight dizygotic twin pairs in which at least one subject was affected by PBC, a concordance rate of 63 % was found, the highest among autoimmune diseases (Selmi et al. 2004). Finally, a role for genetics in PBC is also suggested by animal models of this disease (Hirschfield and Invernizzi 2011), being most of them spontaneous models due to a number of different genetic changes.

Non-HLA Associations and PBC

A large number of classical case-control studies tried to identify genes with a role in disease susceptibility and progression by evaluating one or few single-nucleotide polymorphisms (Hirschfield and Invernizzi 2011). Because of the autoimmune nature of PBC, most of these genes were already implicated in other autoimmune disorders and/or code for immune-related molecules, such as cytotoxic T lymphocyte antigen-4 (CTLA-4); tumor necrosis factor (TNF); vitamin D receptor; caspase 8; toll-like receptors; interleukins (ILs) 1, 2, and 10; and numerous cytokine and chemokine receptors (Hirschfield and Invernizzi 2011). However, such approaches have led to very few insights into the genetic

basis of PBC, mainly for small sample size and lack of solid replication. The data related to CTLA-4 gene association studies provide a paradigmatic example of a long list of studies performed with a long story of contrasting evidences and a not yet clear answer. Classical candidate gene studies with appropriate size and replication should only focus on investigating variant frequencies in different geographical areas, on dissecting interaction between risk loci, and on risk loci influencing outcomes, symptoms, and treatment response.

Role of Sex Chromosome Defects

It has been proposed that the presence of sex chromosome defects might explain both the genetic predisposition to the disease and the female preponderance in PBC (Bianchi et al. 2012). Indeed, it has been reported an age-dependent enhanced monosomy X in the peripheral white blood cells of women with PBC (Invernizzi et al. 2004), we later demonstrated that one X chromosome is preferentially lost (Miozzo et al. 2007), and finally that epigenetic factors influencing PBC onset are more complex than methylation differences at X-linked promoters (Mitchell et al. 2011).

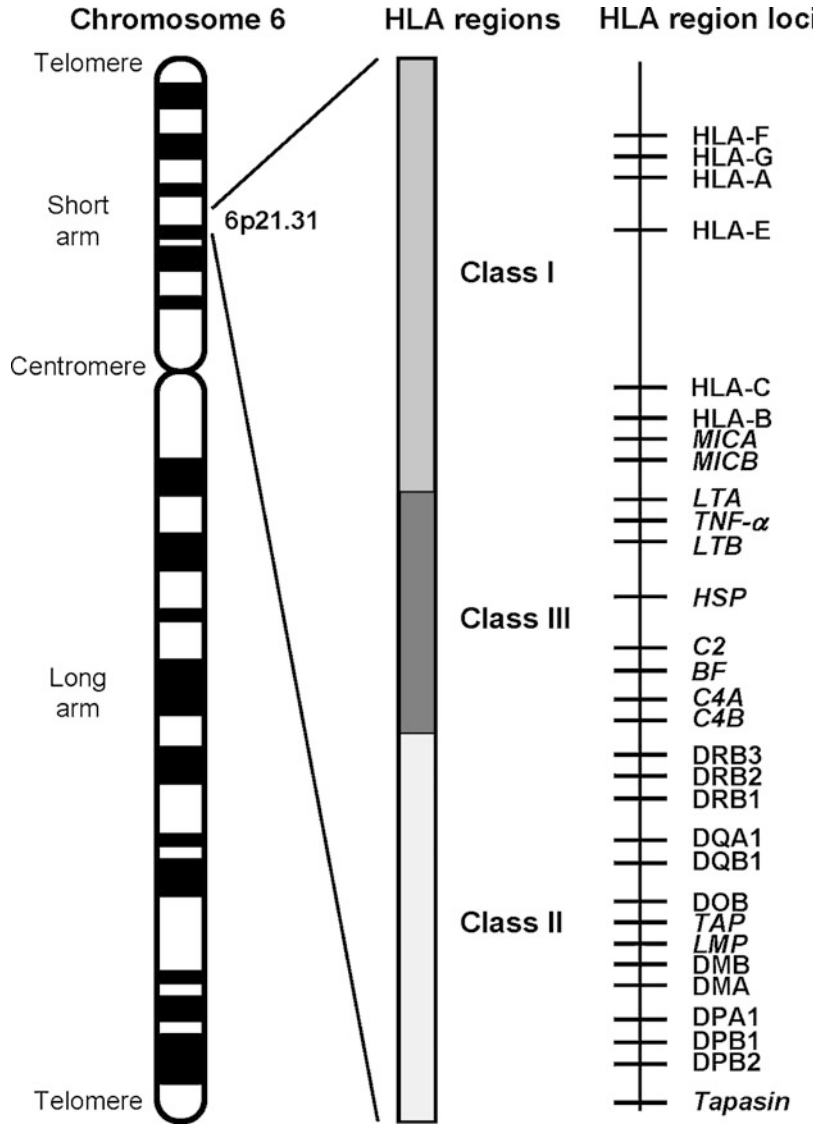
Human Leukocytes Antigen (HLA) Associations and PBC

The human leukocyte antigen (HLA), located in the major histocompatibility complex, is one of the most widely studied regions in human genome because it contains important genetic information of many complex genetic diseases (Invernizzi 2011) (Fig. 1). The role of HLA genes have yet to be fully dissected, but it is known that HLA genes encode cell-surface molecules that, by means of peptides presentation, mediate immunological events, such as cellular immune responses to tumors and pathogens, or definition of self-tolerance. Similar to other genetically complex diseases (Invernizzi 2011), HLA has been extensively studied in PBC, but for a long time data have suggested only a low risk conferred by the HLA DRB1*08 allele (Invernizzi 2011). The reason was likely because early studies manifested limitations such as insufficient statistical power and lack of careful matching between cases and controls and because multiple replications have rarely been carried out.

Only recently the story began to change when, in order to overcome these flows, it has been evaluated the HLA variants in the largest PBC series ever reported (Invernizzi et al. 2008, 2005), thus showing that PBC susceptibility is associated not only with the HLA DRB1*08 allele but also with the protective DRB1*11 and DRB1*13 alleles. A finding later confirmed in other geographical areas (Donaldson et al. 2006). Interestingly, because these protective alleles influence the penetrance of a number of infectious agents, these findings support the infectious theory in PBC origin. However, the interest for HLA genes in PBC arising from these studies was overcome by the three recent genome-wide association studies (GWAS) in PBC which identified the HLA region as the strongest associations (Hirschfield et al. 2009; Liu et al. 2010; Mells et al. 2011). Even more interesting a recent study was able to better define the association of PBC with HLA, by genotyping 676 Italian cases and 1440 controls with dense single-nucleotide polymorphisms (SNPs) for which classical HLA alleles and amino acids were imputed (Invernizzi et al. 2012) (Fig. 2). Interestingly, not only has it been demonstrated that the HLA signals can be attributed to classical DRB1 and DPB1 genes, but it has been provided evidences by conditional analyses supporting a predominant role of DRB1 (mostly *08, *11 and *14) and the independent association of DPB1 (Invernizzi et al. 2012) (Fig. 3).

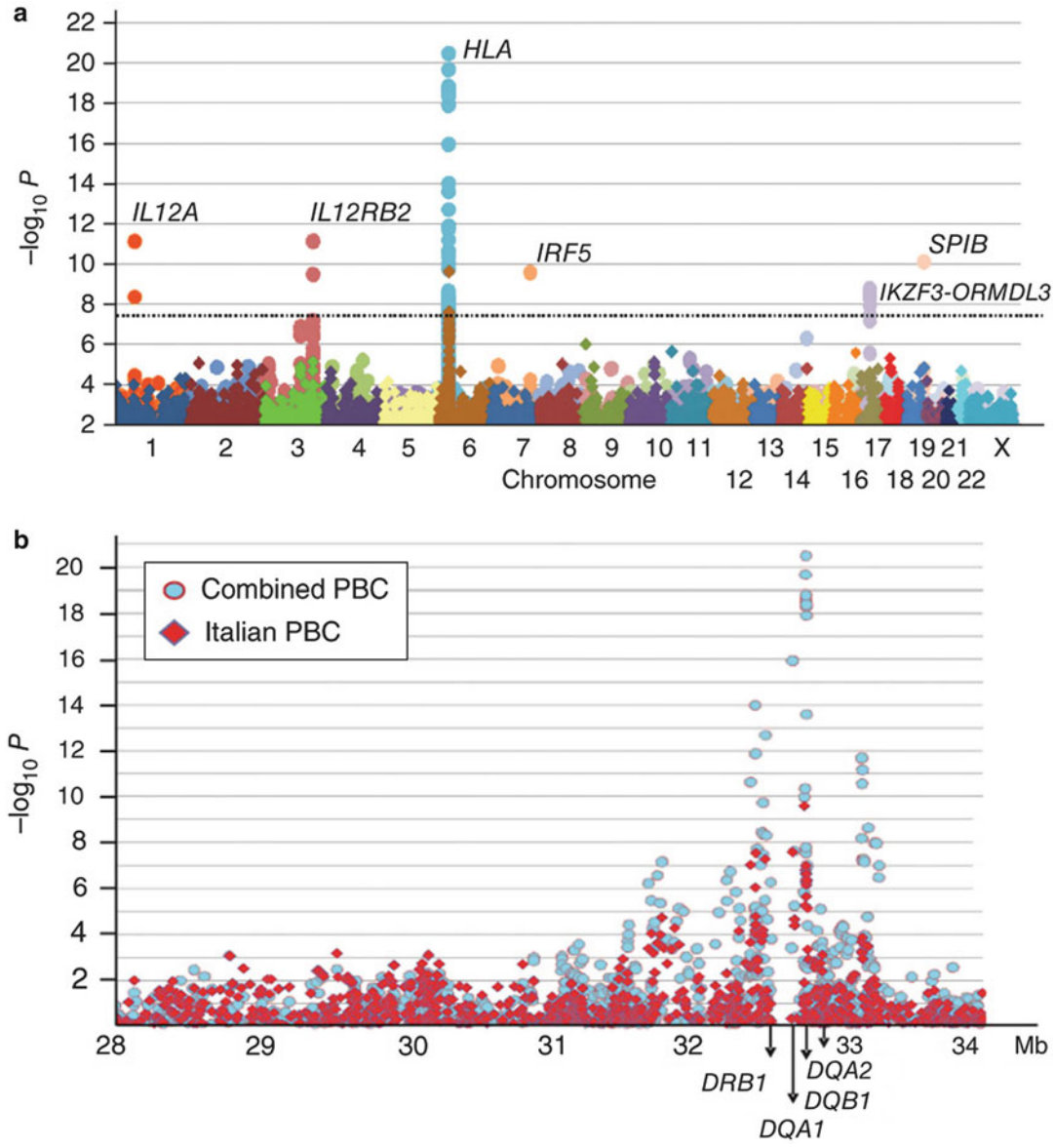
Hints from GWAS in PBC

Since the recent completion of the human genome sequence and thanks to impressive advances in molecular technology, the field of human genetics has recently changed, and we are now witnessing an explosion of new information about the allelic architecture of PBC as well as of many other human complex diseases (Hirschfield and Invernizzi 2011). The three GWAS in PBC identified a number of non-HLA loci, with plausible candidate genes that indicate

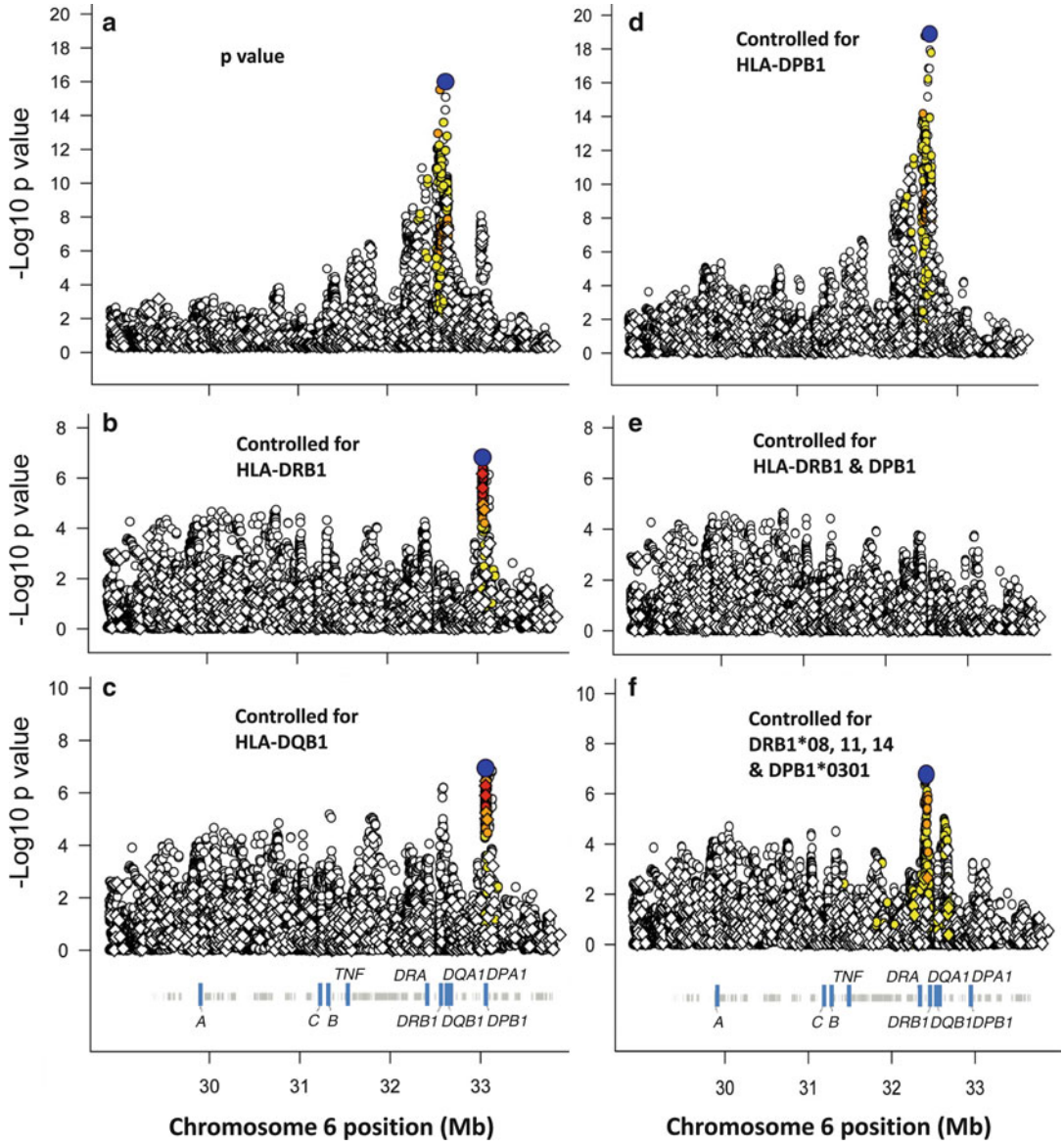


PBC Genetics, Fig. 1 *Human leukocyte antigen (HLA) complex on human chromosome 6.* The small region (6p21.31) in the short arm of chromosome 6 is conventionally divided into three regions (class I, II, and III) and contains many loci that are involved in inflammatory responses. Some of them are shown. Abbreviations: *MICA* major histocompatibility complex class I chain genes A; *MICB* major histocompatibility complex class I chain genes B; *LTA* lymphotoxins A; *TNF-a* tumor necrosis factor-*a*; *LTB* lymphotoxins B; HSP heat-shock protein; *C2* complement component 2; *BF* complement factor B; *C4A* and *C4B* complement components 4A and 4B, respectively; *TAP* transporter associated with antigen processing; *LMP* large multifunctional protease; Tapasin TAP-binding protein (Reproduced with permission of the Hepatology; © 2012. All rights reserved)

the involvement of the innate and adaptive immune systems in the etiopathogenesis of PBC (Hirschfield et al. 2009; Liu et al. 2010; Mells et al. 2011). The first GWAS was performed in cases from North America (Hirschfield et al. 2009), then more solid data were provided by combining datasets from the North American GWAS with a separate Italian GWAS (Liu et al. 2010), and finally a UK GWAS was reported (Mells et al. 2011). Taken together, these findings support the role for the TNF, TLR, and NF-kB pathways, and among the associations consistently reported are those with the IL12A and IL12RB2 loci, the gene encoding interferon regulatory factor 5 (IRF5), the gene encoding the SPi-B transcription factor (SPIB) as well as two other loci, the gene encoding the IKAROS family zinc finger 3 (IKZF3), and that encoding ORM1-like 2 (ORMDL3) also implicated in risk for other autoimmune diseases. Suggestive associations were also observed between PBC and DENND1B and the signal transducer and



PBC Genetics, Fig. 2 *Results of genome-wide association tests for PBC*. The ordinate shows the level of significance for each SNP along each chromosome (**a**) or for the HLA region on chromosome 6 (**b**). The Italian PBC subset (*diamond symbols*) and combined European dataset (*circle symbols*) are shown. The *dashed line* corresponds to $P = 5 \times 10^{-8}$. For HLA, the strongest association was for rs7774434 for both the Italian alone ($P = 2.05 \times 10^{-11}$, odds ratio = 1.74) and for the combined dataset ($P = 1.31 \times 10^{-27}$, odds ratio = 1.71). Abbreviations: HLA-DQB1 denotes the gene encoding HLA class II DQ β-chain 1; IL12A, the gene encoding interleukin-12α; IL12RB2, the gene encoding interleukin-12 receptor β2; IRF5, the gene encoding interferon regulatory factor 5; SPIB, the gene encoding the SPi-B transcription factor; IKZF3, the gene encoding the IKAROS family zinc finger 3; and ORMLD3 encoding ORM1-like 2 (Reproduced with permission of the Nature Genetics; © 2010. All rights reserved)



PBC Genetics, Fig. 3 *Analysis of the HLA region association signals in PBC*. In each panel, the symbols show the strength of the association signal (ordinate) for the corresponding position (Mb, HG19) on chromosome 6 (abscissa). For panel **A**, the *p* value before conditioning is shown. For panels **B–F**, the *p* values are shown after conditioning on the HLA determinant(s) indicated in the panel. The *blue color-coded symbols* denote the strongest associated marker with *p* value $<10^{-6}$, and the other markers are color coded to indicate marker LD with the strongest associated marker: markers with strong LD ($r^2 > 0.8$) (*red*), moderate LD ($r^2 > 0.5$) (*orange*), weak LD ($r^2 > 0.2$) (*yellow*), and little or no LD (*open symbols*) are shown. The SNPs with the strongest associations were rs115721871 at bp 32653792 (panels **A** and **D**), rs9277558 at bp 33056711 (panels **B** and **C**), and rs9268668 at bp 32413889 (panel **F**) (Reproduced with permission of the Genes and Immunity; © 2012. All rights reserved)

activator of transcription 4 (STAT4), two other loci associated with other autoimmune conditions. It is to note that the list of associate genes is still growing and the most recent UK GWAS identified novel associations between PBC and loci, such as NFKB1, IL7R, CD80, CXCR5, and TNFAIP2 (16). This suggests caution and the reasonable need to focus our future research

studies, for example, to rare variants or to copy number variants or to gene expression. A second observation is the impressive consistency among the findings of these three GWAS, thus indicating the presence of a common genetic pattern for PBC. In the future it will be important to replicate the reported associations also in non-European populations. Indeed, a recent study from Japan failed to confirm some GWAS-associated variants (Tanaka et al. 2011).

Conclusions

It is currently believed that the development of PBC, as well as of most of complex diseases, requires that an environmental factor, particularly an infection, initiates an autoimmune reaction in a genetically predisposed individual. However, although strongly implicated by family and twin studies, no specific genetic factors involved in susceptibility to PBC were identified. Up to date, we tried to discover autoimmune risk variants for PBC pathogenesis, but further studies will allow to identify more of the common and uncommon gene variants associated with disease and additional genetic variants that explain sub-phenotypes of PBC and its outcome. Taken together this has the potential to allow the identification of novel therapeutic options.

Cross-References

- ▶ Environment and Autoimmunity
- ▶ Epigenetics in Autoimmunity
- ▶ Primary Biliary Cirrhosis, Overview
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Bianchi I, Lleo A, Gershwin ME, Invernizzi P. The X chromosome and immune associated genes. J Autoimmun. 2012;38:J187–92.

Donaldson PT, Baragiotta A, Heneghan MA, Floreani A, Venturi C, Underhill JA, Jones DE, et al. HLA class II alleles, genotypes, haplotypes, and amino acids in primary biliary cirrhosis: a large-scale study. Hepatology. 2006;44:667–74.

Gershwin ME, Selmi C, Worman HJ, Gold EB, Watnik M, Utts J, Lindor KD, et al. Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. Hepatology. 2005;42:1194–202.

Hirschfield GM, Invernizzi P. Progress in the genetics of primary biliary cirrhosis. Semin Liver Dis. 2011;31:147–56.

Hirschfield GM, Liu X, Xu C, Lu Y, Xie G, Gu X, Walker EJ, et al. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. N Engl J Med. 2009;360:2544–55.

Invernizzi P. Human leukocyte antigen in primary biliary cirrhosis: an old story now reviving. Hepatology. 2011;54:714–23.

Invernizzi P, Miozzo M, Battezzati PM, Bianchi I, Grati FR, Simoni G, Selmi C, et al. Frequency of monosomy X in women with primary biliary cirrhosis. Lancet. 2004;363:533–5.

Invernizzi P, Selmi C, Poli F, Bianchi I, Rosina F, Floreani A, Frison S, et al. HLA-DRB1 polymorphisms in 676 Italian patients with primary biliary cirrhosis and 2028 matched healthy controls. A nationwide population-based case-control study. Hepatology. 2005;42:462A.

Invernizzi P, Selmi C, Poli F, Frison S, Floreani A, Alvaro D, Almasio P, et al. Human leukocyte antigen polymorphisms in Italian primary biliary cirrhosis: a multicenter study of 664 patients and 1992 healthy controls. Hepatology. 2008;48:1906–12.

Invernizzi P, Selmi C, Gershwin ME. Update on primary biliary cirrhosis. Dig Liver Dis. 2010;42:401–8.

Invernizzi P, Ransom M, Raychaudhuri S, Kosoy R, Lleo A, Shigeta R, Franke A, et al. Classical HLA-DRB1 and DPB1 alleles account for HLA associations with primary biliary cirrhosis. Genes Immun. 2012;13:461–8.

Jones DE, Watt FE, Metcalf JV, Bassendine MF, James OF. Familial primary biliary cirrhosis reassessed: a geographically-based population study. J Hepatol. 1999;30:402–7.

Liu X, Invernizzi P, Lu Y, Kosoy R, Bianchi I, Podda M, Xu C, et al. Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. Nat Genet. 2010;42:658–60.

Mells GF, Floyd JA, Morley KI, Cordell HJ, Franklin CS, Shin SY, Heneghan MA, et al. Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. Nat Genet. 2011;43:329–32.

Miozzo M, Selmi C, Gentilin B, Grati FR, Sirchia S, Oertelt S, Zuin M, et al. Preferential X chromosome loss but random inactivation characterize primary biliary cirrhosis. Hepatology. 2007;46:456–62.

Mitchell MM, Lleo A, Zammataro L, Mayo MJ, Invernizzi P, Bach N, Shimoda S, et al. Epigenetic investigation of variably X chromosome inactivated

genes in monozygotic female twins discordant for primary biliary cirrhosis. Epigenetics. 2011;6:95–102.
Selmi C, Mayo MJ, Bach N, Ishibashi H, Invernizzi P, Gish RG, Gordon SC, et al. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. Gastroenterology. 2004;127:485–92.
Tanaka A, Invernizzi P, Ohira H, Kikuchi K, Nezu S, Kosoy R, Seldin MF, et al. Replicated association of 17q12-21 with susceptibility of primary biliary cirrhosis in a Japanese cohort. Tissue Antigens. 2011;78:65–8.

Pemphigus

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Subcorneal Blistering Diseases

Pemphigus Foliaceus

Synonyms

Fogo selvagem (endemic form in South America); Superficial pemphigus

Introduction/Definition

The term "pemphigus" once represented most of the bullous diseases of the skin, but they have been reclassified with advances in diagnostic testing. Pemphigus now refers to a disease with several subgroups that are characterized by autoantibodies of the IgG type which are directed against epidermal cadherins, a family of calcium-dependant cell-cell adhesion glycoproteins. Cell-cell adhesion is mediated by the classic cadherins (E-, P-, and N-cadherins) and the desmosomal cadherins (desmogleins and desmocollins) that interact with plaque proteins α- and β-catenin, as well as with plakoglobin.

Pemphigus vulgaris and pemphigus foliaceus are the two primary subtypes, with unique target antigens (desmoglein-3 in vulgaris and desmoglein-1 in foliaceus) that cause a difference in the level of cleavage in the epidermis. The clinical phenotype correlates with the target antigen. However, cases may not show this traditional relationship, which indicates that antidesmoglein antibodies are not the sole effectors in pemphigus (Herrero-Gonzalez et al. 2006).

Accounting for 10-20 % of pemphigus cases and (unlike pemphigus vulgaris) which affects children and young adults, pemphigus foliaceus is usually sporadic, but endemic cases have been reported in Tunisia and Brazil (Diaz et al. 1989). HLA types D1, DRB1, DR4, and DQ1 have been linked to the development of this disorder (Moraes et al. 1997).

Clinical

The blistering in pemphigus foliaceus, which is less severe than that observed in pemphigus vulgaris, is high in the epidermis, either just beneath the stratum corneum or in the granular layer. Intact, flaccid blisters are rarely seen, with widespread scaling and well-demarcated, crusted erosions being more common. Nikolsky's sign, which is blistering induced by rubbing the skin, is positive in pemphigus foliaceus. There is little mucosal involvement in pemphigus foliaceus as the principal target antigen, desmoglein-1, is only weakly expressed in mucosa (Shirakata et al. 1998). Pemphigus foliaceus is a chronic condition with appreciable morbidity but is rarely fatal, possibly owing to the superficiality of the lesions and lack of mucosal involvement.

Cleavage at desmoglein-1 is also observed with exfoliative toxins from *Staphylococcus aureus* which lead to (clinically and histopathologically similar) bullous impetigo and staphylococcal scalded skin syndrome.

By histopathologic examination, acantholytic cells (separated keratinocytes with lost cell-cell adhesion) may be difficult to detect in pemphigus foliaceus owing to the fragility of blisters but may be found on the blister roof. A loss of desmosomes is observed with dyskeratosis (abnormal keratinization) and detachment of keratin tonofilaments.

Pathophysiology

In pemphigus foliaceus, pathogenic autoantibodies target desmoglein-1 (Dsg-1), which is a 160 kDa desmosomal cadherin. Antibodies

recognize epitopes of the extracellular aminoterminal domain, with the specific site differing between cases. A long cytoplasmic domain distinguishes Dsg-1 from other epithelial cadherins, with this domain mediating the interaction with the 85 kDa plakoglobin and other intracellular adhesion proteins. The pathogenic autoantibodies are largely of the IgG4 type, with titers correlating to the activity and extent of disease. Rarely, antibodies to desmosomal plakins (intracellular desmosomal plaque proteins) have also been identified in patients with pemphigus foliaceus but their relevance is unknown (Jiao & Bystryn 1998).

Direct IF shows positivity around keratinocytes throughout the epidermis, but sera bind more avidly to desmosomes in the upper rather than in the lower epidermis – the opposite is seen with pemphigus vulgaris (Shimizu et al. 1995). C3 may be present, but complement is not required for blistering. A moderate number of acute inflammatory cells and mediators are observed, including eosinophils and plasminogen activator. High concentrations of plasminogen further contribute to cellular acantholysis.

By enzyme-linked immunosorbent assay (ELISA), some reports have shown that sera from patients with pemphigus foliaceus have autoantibodies directed against desmoglein-3, desmocollin 1, and desmocollin 2, but positivity is rare and the relevance is unknown. Some cases of foliaceus that are positive for antidesmoglein-3 antibodies do evolve into clinical pemphigus vulgaris.

Autoantibody production is T-cell dependant.

Pemphigus Erythematosus

Synonym

Senear-Usher syndrome

Introduction/Definition

Pemphigus erythematosus is a variant of pemphigus foliaceus, which shows immunopathological features of both pemphigus and systemic lupus erythematosus (American & Ahmed 1985).

Clinical

Photosensitive lesions on the trunk are similar to those seen in pemphigus foliaceus, and scaly, erythematous plaques over the nose and cheeks may resemble the malar distribution of cutaneous lupus erythematosus. Progression to systemic lupus erythematosus is rare, but pemphigus erythematosus may be associated with myasthenia gravis or thymomas, an observation that is similar to the paraneoplastic form of pemphigus.

Histopathologic features of pemphigus erythematosus are the same as pemphigus foliaceus.

Pathophysiology

The blistering process in pemphigus erythematosus is similar to that in pemphigus foliaceus. Pathogenic IgG autoantibodies target the pemphigus foliaceus antigen desmoglein-1, and circulating antinuclear antibodies (ANA) also are present. Direct IF shows intercellular IgG and C3 in the epidermis, as well as a granular deposition of IgG and C3 at the BMZ (Gomi et al. 1999).

Drug-Induced Pemphigus

Pemphigus foliaceus and vulgaris have also been reported to be exacerbated or induced by radiotherapy, thermal or electrical injury, and numerous drugs. Links to penicillamine, captopril, and other ACE (angiotensin converting enzyme) and ARB (angiotensin II receptor) inhibitors are well documented. Other cases have been linked with chemotherapy, especially with fludarabine. It is unclear whether these cases are truly a result of the chemotherapeutic agent rather than the affiliated malignant condition and paraneoplastic pemphigus.

Intraepidermal Blistering Diseases

Pemphigus Vulgaris

Introduction/Definition

Pemphigus vulgaris, which accounts for roughly 70 % of all cases of pemphigus and affects predominantly the middle aged and elderly, may be the most common autoimmune bullous disease in Eastern countries but is less common in the West. Certain MHC class II genotypes are more frequent in pemphigus vulgaris patients, with

alleles of HLA-DR4, DRB1 (*0402 and 1401), and DQB1*0503 appearing to predispose patients to disease. This effect may be due to structural differences in peptide binding sites that influence antigen presentation and T-cell recognition.

There are two principal subtypes of pemphigus vulgaris – the mucosal-dominant form and the mucocutaneous form. Mucosal-dominant cases may evolve to more mucocutaneous forms or from pemphigus vulgaris to pemphigus foliaceus (rarely vice versa) with correlating shifts in antibody reactivity and specificity to different desmoglein isoforms (Harman et al. 2002). Despite some homology between antigens, antibodies against desmoglein-1 and desmoglein-3 are not thought to be cross-reactive. Passive transfer of maternal IgG antibodies across the placenta may induce transient blisters in newborns. This effect is rarely seen in mothers with pemphigus foliaceus.

It is possible for pemphigus and bullous pemphigoid to coexist.

Clinical

In pemphigus vulgaris, the level of cleavage is lower in the epidermis when compared to that of pemphigus foliaceus, owing to the suprabasal predilection for the pemphigus vulgaris antigen desmoglein-3 (Dsg-3). In contrast to the target antigen of pemphigus foliaceus (Dsg-1), Dsg-3 is also more strongly expressed on the scalp and in the buccal mucosa. This observation accounts for the much more significant degree (50-70 % of cases) of mucosal involvement in pemphigus vulgaris (Shirakata et al. 1998). In patients with autoantibodies due solely to Dsg-3, lesions and acantholysis tend to be limited to mucous membranes where the relative lack of Dsg-1 is unable to compensate and maintain intercellular adhesion (Amagai et al. 1999; Ding et al. 1997). These patients represent the mucosal-dominant form of pemphigus vulgaris. Patients with the mucocutaneous form have autoantibodies to both Dsg-3 and Dsg-1 and clinically present with more severe and widespread blistering of the skin and mucous membranes. The disease is typically widespread and has a penchant for the groin, axillae, face, scalp, and pressure points.

Nail dystrophy, subungual hematoma, and acute paronychias have been reported, but nail involvement is not common.

Basal cells lose lateral adhesion and separate from each other but remain attached to the basement membrane and resemble a "row of tombstones" on histopathologic examination. Keratinocyte necrosis is not seen.

Pathophysiology

The target antigen of Th1 and Th2 pathogenic autoantibodies is desmoglein-3 (Dsg-3), which is a 130 kDa desmosomal cadherin on the keratinocyte surface. Dsg-3 is found primarily in the lower epidermis where it functions in mediating intercellular adhesion (Hertl 2000). Antibodies may be of either (or both) subclasses IgG1 or IgG4, with those of IgG4 thought to be more pathogenic. As with other blistering disorders, disease activity typically correlates with antibody titer. Antibody binding triggers intracellular signaling processes through phosphorylation of EGF receptors and downstream substrates p38 mitogen-activated protein kinase and the Fas apoptotic cascade. After an initial widening of the intercellular space, the tonofilaments of the intracellular cytoskeleton retract around the nucleus causing keratinocyte shrinkage, and acantholysis occurs. However, the specific mechanisms of acantholysis in pemphigus vulgaris are not yet fully understood.

Pemphigus autoantibodies also fix complement to the keratinocyte surface which enhances pathogenicity, although complement is not required for disease (Kawana et al. 1989). In addition to complement fixation, pemphigus antibodies also lead to T-cell activation and recruitment through release of inflammatory mediators interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), thromboxane B2, leukotriene B4, and plasminogen activator (Grando et al. 1989; Zillikens et al. 1993).

The pathogenicity of anti-Dsg-3 antibodies has been well described, but autoantibodies to desmocollins, desmoplakin, e-cadherin, and non-cadherin targets in pemphigus vulgaris patients have also been reported. The relevance of these findings is unclear.

Direct IF shows IgG deposition on keratinocyte surfaces throughout the epidermis, with indirect IF detecting circulating autoantibodies in 80 % of cases. Rarely, IgA and/or IgM are present. A more robust staining in the lower epidermis in vulgaris patients may differentiate the direct IF pattern from pemphigus foliaceus. This reflects the higher density of Dsg-3 on desmosomes in this region.

Pemphigus Vegetans

Synonym

Vegetating bullous pemphigoid

Introduction/Definition

A rare variant of pemphigus vulgaris, pemphigus vegetans is defined by the formation of vegetating granulations mostly in flexural lesions and within skin folds. Pemphigus vegetans has two subtypes, the mild Hallopeau form and the more severe Neumann form, although these divisions are now of mainly historic interest.

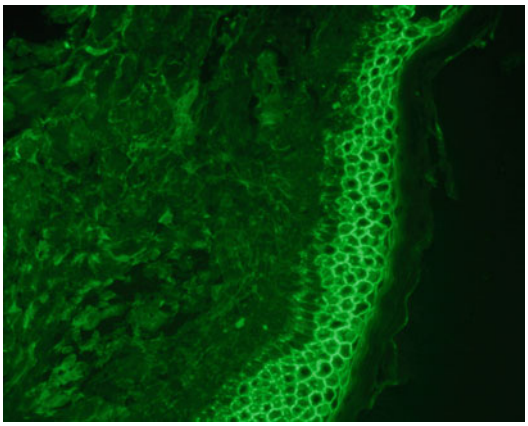
Clinical

Affecting primarily middle-aged adults, lesions show the classic acantholysis of other forms of pemphigus but are more papillomatous and hyperkeratotic. In the Neumann type, vesicles and bullae rupture and form easily bleeding hypertrophic and granular erosions. The erosions evolve into purulent, vegetating lesions that eventually become hyperkeratotic, dry, and fissured. The Hallopeau type differs in that pustules rather than vesicles characterize early lesions. However, pustules are observed in more advanced lesions of both Hallopeau and Neumann types.

Pathophysiology

Both forms of pemphigus vegetans show antibodies that target Dsg-3, the same antigen as pemphigus vulgaris. The Hallopeau type may also show reactivity with desmocollins 1 and 2. A dense infiltrate of neutrophils and eosinophils is observed along with complement fixation, all of which contribute to the purulent and vegetative nature of these lesions.

As with other forms of pemphigus, direct IF shows intercellular IgG (Fig. 1) and sometimes C3 (Fig. 2), with indirect IF detecting circulating intercellular antibodies in most patients.



Pemphigus, Fig. 1 Direct immunofluorescence figures. Intercellular IgG surrounding keratinocytes throughout the epidermis in pemphigus vulgaris

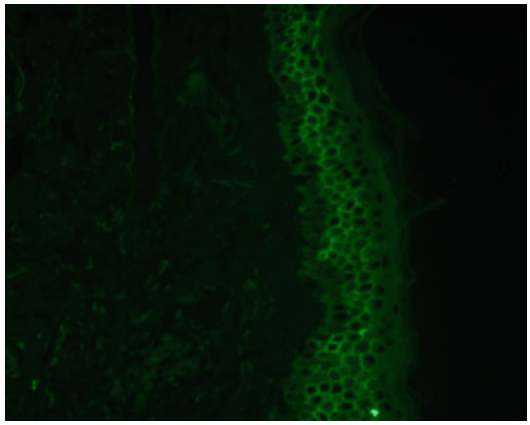
Paraneoplastic Pemphigus

Introduction/Definition

Paraneoplastic pemphigus (PNP) is a distinct form that is associated with an underlying neoplasm, most frequently B-cell lymphomas and other hematologic and lymphoproliferative malignant conditions such as chronic lymphocytic leukemia and giant follicular hyperplasia (Castleman's tumor). Association with thymomas and several other types of carcinoma and sarcoma has also been reported (Anhalt et al. 1990). Blistering may be the initial finding, but typically the neoplasm is already detected at the time of PNP diagnosis (Robinson et al. 1999). PNP is a devastating illness with much more profound morbidity than is observed in other forms of pemphigus; mortality often is attributed to the underlying malignant conditions as well. Many patients succumb to sepsis, gastrointestinal bleeding, and respiratory or multi-organ failure (Robinson et al. 1999; Anhalt 1997). Low-grade disease may improve after treatment of the associated neoplasm.

Clinical

Clinically overlapping with erythema multiforme (EM) and lichen planus pemphigoides (LPP), patients present with more severe and refractory



Pemphigus, Fig. 2 C3 is often present surrounding keratinocytes throughout the epidermis in pemphigus vulgaris

mucosal erosions (especially oral stomatitis) and polymorphous cutaneous findings, which range from blisters and erosions to palmoplantar target lesions (Anhalt et al. 1990; Robinson et al. 1999; Zillikens & Brocker 1994). PNP shows a distinct combination of keratinocyte necrosis (resembling EM), suprabasilar acantholysis (resembling pemphigus vulgaris), and a possible lichenoid eruption (resembling LPP). PNP is a multisystem disease, and beyond the skin, autoantibody deposits may also be found in the kidney, bladder, respiratory and digestive tract epithelia, and in both smooth and striated muscle.

Considerable variety exists histopathologically in cutaneous lesions of paraneoplastic pemphigus. Suprabasilar acantholysis, individual keratinocyte necrosis, basal cell liquifactive degeneration, and a band-like dense lymphocytic infiltrate in the upper dermis may all be seen.

Pathophysiology

The antigenic target(s) and humoral response of PNP are more heterogeneous and less defined than those in other forms of pemphigus, with pathogenic antibodies of principally the IgG1 subclass targeting linker subdomains of envoplakin (210 kDa) and periplakin (190 kDa) and also other desmosomal plakins (intracellular desmosomal plaque proteins) which include desmoplakin-1 (250 kDa), desmoplakin-2 (215 kDa), plectin (500 kDa), and the hemidesmosomal bullous pemphigoid antigen (230 kDa). The broad specificity of autoantibodies, combined with the addition of cell-mediated cytotoxicity to the humoral effect, causes the widespread systemic effects associated with PNP.

Antiplakin antibodies are not specific to PNP and have also been identified in patients with pemphigus foliaceus, bullous pemphigoid, toxic epidermal necrolysis, and erythema multiforme.

Direct IF shows IgG with or without C3 deposited along epidermal cell surfaces and variably along the basement membrane zone. Indirect IF shows autoantibodies that also react with simple or transitional epithelia, unlike those of classic pemphigus vulgaris.

References

Amagai M, Tsunoda K, Zillikens D, Nagai T, Nishikawa T. The clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile. J Am Acad Dermatol. 1999;40:167–70.

American M, Ahmed A. Pemphigus erythematosus. Int J Dermatol. 1985;24:16–25.

Anhalt GJ. Paraneoplastic pemphigus. Adv Dermatol. 1997;12:77–96; discussion 97.

Anhalt GJ, et al. Paraneoplastic pemphigus. An autoimmune mucocutaneous disease associated with neoplasia. N Engl J Med. 1990;323(25):1729–35.

Diaz LA, et al. Endemic pemphigus foliaceus (fogo selvagem). I. Clinical features and immunopathology. J Am Acad Dermatol. 1989;20(4):657–69.

Ding X, Aoki V, Mascaro Jr JM, Lopez-Swiderski A, Diaz LA, Fairley JA. Mucosal and mucocutaneous (generalized) pemphigus vulgaris show distinct autoantibody profiles. J Invest Dermatol. 1997;109:592–6.

Gomi H, et al. Pemphigus erythematosus: detection of anti-desmoglein-1 antibodies by ELISA. Dermatology. 1999;199(2):188–9.

Grando SA, Glukhenky B, Drannik GN, Epshtein EV, Kostromin AP, Korostash TA. Mediators of inflammation in blister fluids from patients with pemphigus vulgaris and bullous pemphigoid. Arch Dermatol. 1989;125:925–30.

Harman KE, Gratian M, Shirlaw PJ, Bhogal BS, Challacombe SJ, Black MM. The transition of pemphigus vulgaris into pemphigus foliaceus: a reflection of changing desmoglein 1 and 3 autoantibody levels in pemphigus vulgaris. Br J Dermatol. 2002;146:684–7.

Herrero-Gonzalez JE, Mascaró JJ, Iranzo P, Herrero C. Atypical pemphigus: discordance between clinicopathological findings and the antigenic profile in four cases. J Cutan Pathol. 2006;33:502–7.

Hertl M. Humoral and cellular autoimmunity in autoimmune bullous skin disorders. Int Arch Allergy Immunol. 2000;122:91–100.
Jiao D, Bystryn JC. Antibodies to desmoplakin in a patient with pemphigus foliaceous. J Eur Acad Dermatol Venereol. 1998;11(2):169–72.
Kawana S, Geoghegan W, Jordon RE, Nishiyama S. Deposition of the membrane attack complex of complement in pemphigus vulgaris and pemphigus foliaceus skin. J Invest Dermatol. 1989;92:588–92.
Moraes ME, et al. An epitope in the third hypervariable region of the DRB1 gene is involved in the susceptibility to endemic pemphigus foliaceus (fogo selvagem) in three different Brazilian populations. Tissue Antigens. 1997;49(1):35–40.
Robinson ND, et al. The new pemphigus variants. J Am Acad Dermatol. 1999;40((5 Pt 1):649–71; quiz 672-3.
Shimizu H, et al. Pemphigus vulgaris and pemphigus foliaceus sera show an inversely graded binding pattern to extracellular regions of desmosomes in different layers of human epidermis. J Invest Dermatol. 1995;105(2):153–9.
Shirakata Y, et al. Lack of mucosal involvement in pemphigus foliaceus may be due to low expression of desmoglein 1. J Invest Dermatol. 1998;110(1):76–8.
Zillikens D, Brocker EB. Paraneoplastic pemphigus. Induction of autoantibodies against structural proteins in the skin. Hautarzt. 1994;45(12):827–33.
Zillikens D, Ambach A, Zentner A, Dummer R, Schüssler M, Burg G, et al. Evidence for cell-mediated immune mechanisms in the pathology of pemphigus. Br J Dermatol. 1993;128:636–43.

PI3K

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Synonyms

Phosphatidylinositol 3-kinase; Phosphoinositide 3-kinase; PI 3-kinase

Definition

PI3Ks are a family of enzymes that can phosphorylate the inositol head group of phosphoinositides (PtdIns) on the D3 position.

History

PI3Ks were initially discovered by Lewis Cantley and colleagues as an enzyme that could phosphorylate membrane inositol lipids on the D3 position (Whitman et al. 1988). In contrast to PtdIns phosphorylated on the 4 and 5 positions, PtdIns phosphorylated on the D3 position was found to be resistant to hydrolysis by phospholipases. PI3K was found to be associated with tyrosine-phosphorylated Src and SV40 middle T antigens, which suggested that it may play a role in cancer, but was also found in complex with insulin and growth factor receptors, suggesting a broader role in growth and development. PI3K activity was also found in complex with Gβγ proteins in neutrophils which indicated a role for PI3K in inflammation. The class III PI3K vacuolar protein sorting 34 (Vps34) was first discovered in a yeast mutant with abnormal vacuoles, whereas the class II PI3K isoforms were identified by homology cloning and completed the collection of 8 PI3K catalytic subunits encoded by mammalian genomes. The discovery that wortmannin specifically inhibits PI3K and the development of PI3K inhibitor LY294002 by Eli Lilly allowed investigators to probe the function of PI3K in cells. PI3Ks are now known to be central signal transducers in fundamental cellular biological processes such as metabolism, migration, differentiation, proliferation, and autophagy. PI3Ks influence many physiological processes and are also important factors that promote cancer, immune-mediated diseases, and metabolic disorders (Engelman et al. 2006).

The PI3K Family

Humans have eight different PI3Ks divided in three classes (Vanhaesebroeck et al. 2010a).

The **class I PI3Ks** p110α, p110β, p110δ, and p110γ preferentially phosphorylate PtdIns(4,5)P_2 to produce PtdIns(3,4,5)P_3. p110α, p110β, and p110δ are bound by a p85 regulatory subunit (or the smaller p55 or p50 variants) which has SH2 domains that bind phosphorylated tyrosines, typically associated with plasma membrane

receptors. P110γ binds a p101 or p84 regulatory subunit that associates with Gβγ subunit of G protein-coupled receptors. As a consequence, many receptors expressed by immune cells can activate one or more of the PI3K isoforms. The p110δ and p110γ isoforms have received much attention by immunologists as these are expressed at much higher levels in white blood cells than in other cell types.

The **class II PI3Ks** C2α, C2β, and C2γ phosphorylate PI or PI4P to produce PI3P or $PI(3,4)P_2$. Little is known about the role of this class of PI3Ks in immunity.

The only **class III PI3K**, Vps34, phosphorylates PI to produce PI3P. Vps34 binds to the Vps15 regulatory subunit. Vps34 is the only PI3K found in yeast. It is therefore likely that the other PI3Ks evolved from this founding member. Vps34 has received much attention in recent years as a central regulator of autophagy – the process in which cells self-digest when faced with inadequate nutrient supply.

Signaling by PI3Ks

Receptors that can engage and activate class I PI3K on immune cells include growth factor receptors, such as colony-stimulating factor 1 (CSF1); antigen receptors, such as the B cell receptor (BCR) and T cell receptor (TCR); costimulatory receptors such as CD28 and inducible costimulator (ICOS); cytokine receptors such as interleukin-2 (IL-2) and IL-4; and various chemokine receptors. Some receptors such as the B cell-activating factor (BAFF) receptor on B cells may engage the PI3K pathway indirectly by stimulating the transcription of proteins that activate PI3K (Okkenhaug and Fruman 2010).

$PtdIns(3,4,5)P_3$ acts as a lipid anchor in the inner leaflet of the plasma membrane. There are more than 300 proteins with PH domains, and it is estimated that 20–100 of these bind preferentially to $PtdIns(3,4,5)P_3$ over other membrane lipids and proteins. Most prominent of the $PtdIns(3,4,5)P_3$-binding proteins is Akt which is the only $PtdIns(3,4,5)P_3$ effector protein also found in some simpler organisms such as worms. Akt becomes recruited to the plasma membrane by $PtdIns(3,4,5)P_3$ where it gets phosphorylated by Pdk1 and by the mammalian target of rapamycin complex 2 (mTORc2). Akt then phosphorylates a number of substrates. Two main downstream pathways have been explored extensively in immune cells: the activation of mTORc1 and the inhibition of Foxo. mTOR is a kinase that regulates protein translation and lipid synthesis and therefore is a central controller of cell growth. The two complexes, mTORc1 and mTORc2, contain the same central mTOR catalytic subunit in association with the regulatory subunits such as Raptor and Rictor, respectively. Foxo is a family of transcription factors that control the expression of genes that promote apoptosis, cell cycle arrest, and, perhaps most pertinently in immune cells, cytokine receptors and receptors involved in cell trafficking (Hawkins et al. 2006; Finlay and Cantrell 2011).

PI3K signaling is antagonized by phosphatases. Pten removes the phosphate at the D3 position of $PtdIns(3,4,5)P_3$, whereas Ship removes the D5 phosphate, and Inpp4 removes the D4 phosphate. Mice lacking Pten in hematopoietic cells present with leukemia and autoimmunity. Mice lacking Ship also present with autoimmunity. Ship has received much attention from immunologists as it is bound by the cytoplasmic immunoreceptor tyrosine-based inhibition motifs (ITIMs) of a number of inhibitory receptors such as FcγRIIB on B cells (Okkenhaug and Fruman 2010; Leslie et al. 2011).

Role of PI3K in Innate Immunity

Neutrophils are among the first cells to arrive at the site of infection. p110γ, which is activated by chemokine receptors, plays an important role in promoting the recruitment of neutrophils to the site of infections. Once there, neutrophils phagocytose bacteria and kill them, in part by generating reactive oxygen species (ROS). In primed human neutrophils, p110γ and p110δ are

sequentially activated to promote a full ROS response when exposed to a bacterial peptide agonist. Antibodies bound by antigen can also elicit a ROS response in neutrophils. Curiously, in this case, p110β is the most important isoform, followed by p110δ. P110γ appears not to control antibody-stimulated ROS production in neutrophils. As neutrophils are major causes of inflammation, inhibiting p110β, p110γ, or p110δ may have beneficial effects in some clinical settings (Hawkins et al. 2006; Kulkarni et al. 2011).

Macrophages and dendritic cells are also important scavengers of bacteria and in addition form an important link between innate and adaptive immunity. In these cell types, PI3Ks appear to antagonize the response to certain bacterial cell membrane constituents, such as lipopolysaccharide (LPS). Thus inhibiting PI3K can enhance the production of cytokines such as IL-12 which is a central mediator of cell-mediated immunity. Consequently, inhibition of PI3K in innate cells can enhance immunity against intracellular pathogens (Okkenhaug and Fruman 2010).

Both p110γ and p110δ have been shown to control various function in natural killer (NK) cells. NK cells are important for the elimination of neoplasms, and p110δ and p110γ can control both the recruitment of NK cells to tumors and, to some extent, the ability of NK cells to kill tumor cells (Kerr and Colucci 2011).

Role of PI3K in Adaptive Immunity

Lymphocytes become fully activated several days after the initial infection. PI3Ks play important roles in the development and function of lymphocytes. P110α and p110δ are essential for B cell development, whereas p110γ and p110δ are essential for T cell development. P110δ plays a very important role in humoral B cell responses, both by regulating the development of certain B cell subsets and by promoting the expansion or survival of specialized T cells that provide essential help for B cells to undergo immunoglobulin class switching and affinity maturation. Indeed, p110δ controls the expression of a number of cytokines produced by CD4 and CD8 T cells. P110δ-deficient mice have fewer regulatory T cells (Treg), and these are less capable of suppressing immune responses than wild-type Treg. Therefore, while inhibition of PI3K is generally thought to suppress immune responses (e.g., antibody response), there are circumstances where the ability of p110δ to suppress Treg function means that, on balance, the immune response of p110δ-deficient mice is enhanced. In most cases, however, the net result of inhibiting p110δ is the suppression of immune function (Okkenhaug and Fruman 2010).

PI3Ks in Autoimmunity and Inflammation

Studies have shown that inhibition of p110γ and/or p110δ (and in one case p110β) can reduce the severity of diseases in mouse models of rheumatoid asthma, arthritis, multiple sclerosis, and systemic lupus erythematosus. Broadly speaking, p110γ has a stronger impact on innate immunity, whereas p110δ inhibition mostly affects adaptive immune responses – however, this is only a rule of thumb – many exceptions are known. To what extent these preclinical findings will be translated to the clinic remains to be determined (Rommel 2010; Vanhaesebroeck et al. 2010b).

PI3Ks as Pharmaceutical Drug Targets

Because PI3Ks are involved in many facets of immune cell function, there is a considerable effort to target these enzymes to treat or cure immune-mediated diseases. PI3Ks are sufficiently devolved from protein and other lipid kinases that it is relatively easy to target this family specifically. Moreover, in recent years, highly selective inhibitors against specific PI3K isoforms have been generated. The p110δ-selective inhibitor CAL-101 was developed by Calistoga which was subsequently taken over by Gilead who now call it GS-1101. GS-1101 has entered phase II/III clinical trials in

chronic lymphocytic leukemia and indolent non-Hodgkin's lymphoma after patients in phase I trials showed promising clinical responses with few obvious side effects (Burger 2011).

Conclusion

The PI3K family controls diverse biological processes of relevance to the immunologist. PI3Ks regulate multiple processes during immune cell development, activation, and migration. In addition, drugs that target selected PI3K isoforms are beginning to show promise in the clinic.

Cross-References

- ▶ Mammalian Target of Rapamycin (mTOR)
- ▶ SH2 Domain-containing Inositol Phosphatase-1 (SHIP)
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Burger JA. Inhibiting B-cell receptor signaling pathways in chronic lymphocytic leukemia. Curr Hematol Malig Rep. 2012(1):26–33. Epub 2011/11/23.

Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet. 2006;7(8): 606–19.

Finlay D, Cantrell DA. Metabolism, migration and memory in cytotoxic T cells. Nat Rev Immunol. 2011;11(2):109–17. Epub 2011/01/15.

Hawkins PT, Anderson KE, Davidson K, Stephens LR. Signalling through class I PI3Ks in mammalian cells. Biochem Soc Trans. 2006;34(Pt 5):647–62.

Kerr WG, Colucci F. Inositol phospholipid signaling and the biology of natural killer cells. J Innate Immun. 2011;3(3):249–57. Epub 2011/03/23.

Kulkarni S, Sitaru C, Jakus Z, Anderson KE, Damoulakis G, Davidson K, et al. PI3Kbeta plays a critical role in neutrophil activation by immune complexes. Sci Signal. 2011;4(168):ra23. Epub 2011/04/14.

Leslie NR, Dixon MJ, Schenning M, Gray A, Batty IH. Distinct inactivation of PI3K signalling by PTEN and 5-phosphatases. Adv Enzyme Regul. 2012;52(1): 205–13. Epub 2011/09/21.

Okkenhaug K, Fruman DA. PI3Ks in lymphocyte signaling and development. Curr Top Microbiol Immunol. 2010;346:57–85. Epub 2010/06/22.

Rommel C. Taking PI3Kdelta and PI3Kgamma one step ahead: dual active PI3Kdelta/gamma inhibitors for the treatment of immune-mediated inflammatory diseases. Curr Top Microbiol Immunol. 2010;346:279–99. Epub 2010/07/02.

Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol. 2010a;11(5):329–41. Epub 2010/04/10.

Vanhaesebroeck B, Vogt PK, Rommel C. PI3K: from the bench to the clinic and back. Curr Top Microbiol Immunol. 2010b;347:1–19. Epub 2010/06/16.

Whitman M, Downes CP, Keeler M, Keller T, Cantley L. Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. Nature. 1988;332(6165):644–6.

PKC-θ

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Synonyms

PKC-θ; Protein kinase C theta

Protein Kinase C Family

Protein kinase C (PKC) is family of serine/threonine kinases that can be divided into three subfamilies (Newton 1997): conventional PKC, including PKCα, β, and γ, which are activated by Ca^{2+} and diacylglycerol (DAG); novel PKC, including PKCδ, θ, η, and ε, in which activation is dependent on DAG but independent of Ca^{2+}; and atypical PKC, including PKCζ and λ, in which activation is independent of both Ca^{2+} and DAG. PKC-θ is predominantly expressed in T cells. Upon T cell activation, PKC-θ specifically translocates to the immunological synapse and mediates T cell receptor (TCR) signaling critical for the regulation of T cell function (Barouch-Bentov et al. 2005; Dustin 2009).

PKC-θ Mediates TCR Signals Required for T Cell Activation

T cells play a critical role in adaptive immune response. Defects or dysregulation of T cell activation or function leads to immunodeficiencies or autoimmunity. Coordinated activation of T cells in response to antigen leads to clonal expansion and differentiation of antigen-specific T cells. T cell activation is controlled by numerous signaling pathways initiated by the TCR and co-stimulatory molecules (Weiss and Littman 1994). Biochemical signals initiated by the TCR determine the specificity of T cell activation and the signaling pathways mediated by co-stimulatory molecules modulate the activation threshold. As a result of antigen binding to the TCR, PKC-θ translocates to the immunological synapse where it mediates TCR signaling pathways (Monks et al. 1998). TCR signaling is initiated by activation of the Src family lymphocyte-specific protein tyrosine kinase (LCK), which leads to recruitment and activation of zeta-chain-associated protein kinase 70 (ZAP70) and consequently of the adaptor proteins linker of activated T cells (LAT), SH2 domain-containing leukocyte protein of 76 kDa (SLP76), and VAV. LAT recruits phospholipase Cγ1 (PLCγ1), which catalyzes the breakdown of phosphatidylinositol 4,5-bisphosphate into inositol triphosphate (IP3), a Ca^{2+} mobilizer, and DAG, the PKC-θ activator (Weiss and Littman 1994). Upon activation by DAG, PKC-θ mediates the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Several adaptor proteins play a critical role in mediating PKC-θ-induced NF-κB activation including membrane-associated guanylate kinase (MAGUK), caspase recruitment domain (CARD)11 (CARMA1), B cell lymphoma 10 (Bcl10), and mucosa-associated lymphoid tissue 1 (MALT1). Together with PKC-θ, these adaptors facilitate the activation of IκB kinase (IKK), which leads to the phosphorylation, ubiquitination, and degradation of the IκBα protein, resulting in the release and translocation of NF-κB into the nucleus, where it stimulates the expression of target genes essential for T cell activation, including IL-2. Studies using PKC-θ-deficient T cells, or cells overexpressing a constitutively active form of PKC-θ or a catalytically inactive form of PKC-θ, have demonstrated that PKC-θ also activates the activator protein 1 (AP-1) signaling pathway (Sun et al. 2000). AP-1 is composed of c-Jun and c-Fos. Although the mechanism by which PKC-θ mediates activation of AP-1 is not completely understood, several studies have provided some insight into this process. Ras and the mitogen-activated protein kinases (MAP kinases), c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and P38 mitogen-activated protein kinase (P38) are all involved in PKC-θ-mediated AP-1 activation (Altman et al. 2000). A PKC-θ-interacting MAP kinase, originally termed Ste20/SPS1-related proline and alanine-rich kinase (SPAK or PASK) (Li et al. 2004), was isolated and shown to be involved in the PKC-θ-mediated activation of AP-1, but not NF-κB. Furthermore, activation of this kinase by TCR/CD28 stimulation was impaired in T cells from *PKC-θ*$^{-/-}$ mice, and SPAK-specific small interfering RNA (siRNA) inhibited PKC-θ-mediated AP-1, but not NF-κB, activation, suggesting that SPAK is responsible for activation of AP1 downstream of PKC-θ. Activation of T cells through the TCR also leads to IP3-mediated elevation of cytosolic $[Ca^{2+}]_i$ by inducing Ca^{2+} influx. Elevated intracellular Ca^{2+} ultimately leads to activation of the phosphatase calcineurin, which dephosphorylates nuclear factor of activated T cells (NFAT), resulting in its translocation into the nucleus. Translocated NFAT cooperates with AP-1 to activate interleukin-2 (IL-2) expression. It was shown that, in the absence of AP-1, NFAT activation could lead to T cell anergy. Several studies have shown that PKC-θ enhances the activation of NFAT by stimulating Ca^{2+} influx as TCR-induced Ca^{2+} influx and NFAT activation are defective in T cells from *PKC-θ*$^{-/-}$ mice (Manicassamy et al. 2006a; Pfeifhofer et al. 2003). It is clear that PKC-θ regulates Ca^{2+} signals via PLCγ1; however, it is not known how PKC-θ stimulates PLCγ1. The tyrosine kinase epithelial (TEK kinase) family member IL2-inducible T cell kinase (Itk) may be the missing link. Itk-deficient T cells display defective

Ca^{2+} influx and PLCγ1 activation. Furthermore, PLCγ1 activity is greatly stimulated by Itk overexpression. Therefore, it is possible that PKC-θ regulates PLCγ1 activation via Itk. Altogether, PKC-θ-mediated TCR signals regulate multiple signaling pathways including NF-κB, AP-1, and NFAT, all of which are critical for T cell activation. Inhibition of PKC-θ is therefore expected to prevent T cell activation by blocking these TCR signaling pathways (Altman et al. 2000; Manicassamy et al. 2006b).

PKC-θ Regulates the Differentiation of Naïve T Cells to Effective T Helpers

Peripheral naïve T cells cannot mediate actual immune responses unless they undergo a differentiation process to become effector T cells. Upon T cell activation, these same T cells can differentiate into inflammatory T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17) or inhibitory T regulatory cells (Treg). A fine balance between inflammatory and inhibitory T cells is required for a functional immune system. Overproduction of inflammatory T cells leads to aggravating autoimmunity, whereas overproduction of Tregs can induce tolerance, and PKC-θ is able to shift the balance between these T cell subtypes. *PKC-*$\theta^{-/-}$ mice display impaired Th2 responses following infection with *Nippostrongylus brasiliensis*, whereas they develop a normal Th1-mediated response against *Leishmania major* (Marsland et al. 2004). In addition, *PKC-*$\theta^{-/-}$ mice are resistant to the induction of experimental autoimmune encephalomyelitis (EAE), due to reduced Th17 responses (Tan et al. 2006). PKC-θ is therefore required to fully develop Th2, Th17, and even certain Th1 responses in vivo. Recently, it was shown that PKC-θ-mediated signals inhibit Treg differentiation via an AKT kinase-forkhead box O1/O3a (FOXO1/3a) pathway (Ma et al. 2012). Transforming growth factor β (TGF-β)-induced Treg differentiation was potentiated by a deletion of the PKC-θ gene or by treatment with a specific PKC-θ inhibitor but inhibited by treatment with phorbol myristate acetate (PMA), a PKC-θ activator, or by CD28 cross-linking. Furthermore, CD28 cross-linking inhibited Treg differentiation in wild-type (WT) T cells, but not in *PKC-*$\theta^{-/-}$ T cells or in WT T cells treated with a PKC-θ inhibitor, suggesting that PKC-θ-mediated CD28 signals are responsible for inhibiting Treg differentiation. Consistently, adoptively transferred naïve *PKC-*$\theta^{-/-}$ T cells formed significantly more Tregs in vivo than WT T cells. Furthermore, enhanced Treg differentiation of *PKC-*$\theta^{-/-}$ T cells was associated with lower AKT kinase activity, and forced expression of an active form of AKT kinase inhibited *PKC-*$\theta^{-/-}$ T cells from differentiating into Tregs, indicating a role for AKT downstream of PKC-θ in the inhibition of Treg differentiation. The downstream targets of AKT kinase-FOXO1/3a were also affected in *PKC-*$\theta^{-/-}$ T cells. Accordingly, overexpression or knockdown of FOXO1/3a promoted or inhibited Treg differentiation, respectively. Therefore, PKC-θ promotes inflammatory effector T cell differentiation via NFAT, AP-1, and NF-κB but inhibits Treg differentiation via an AKT-FOXO1/3a pathway. Taken together, PKC-θ is able to control T cell-mediated immune responses by shifting the balance between stimulatory effector T cells and inhibitory Treg cells. A recent study also showed that PKC-θ-mediated signals inhibit the suppressive function of Treg (Zanin-Zhorov et al. 2010). Therefore, PKC-θ regulates T cell-mediated immunity by affecting both the inflammatory and inhibitory arms of the immune system.

PKC-θ in Allograft Rejection

Solid organ transplantations that benefit end-stage organ failure patients are severely limited by the occurrence of rejection. Alloreactive T cells are critical targets for tolerance induction since they mediate immune responses required for rejection. The alloreactive T cell pool is very large, which explains why immune responses against allografts are at least two orders of magnitude stronger than the immune responses

against a specific antigen. Therefore, long-term tolerance to allografts is extremely difficult to establish. Alloreactive T cells can be tolerized through anergy, suppression, and deletion. Anergy is an unresponsive state of T cells, which can be overcome by exogenous IL-2. Suppression is usually mediated by suppressor cells, such as Foxp3-expressing Tregs. However, these two mechanisms are unlikely to change the size of the alloreactive T cell pool. A similar problem exists with the use of cyclosporin A (CsA), the most successful immunosuppressive drug used clinically so far. However, CsA also prevents apoptosis of alloreactive T cells by inhibition of T cell activation, resulting in the accumulation of large amounts of alloreactive T cells that can destroy allografts once immunosuppressive drugs are discontinued. Therefore, the prevention of allograft rejection usually requires transplant recipients to take lifelong immunosuppressive drugs, which can result in other complications including infections and malignancy. In contrast, deletion induces tolerance by decreasing the number of alloreactive T cells via apoptosis. Deletion can occur both in the thymus and peripheral lymphoid tissues. In the periphery, activated T cells are deleted by two different forms of apoptosis: activated induced cell death (AICD) and passive cell death. AICD eliminates clonally expanded T cells via tumor necrosis factor (TNF) family death receptors, such as Fas/FasL, which are largely independent of B cell lymphoma-extra large (Bcl-$x_{L)}$, whereas passive cell death can be inhibited by Bcl-x_L. Activated T cells upregulate Bcl-x_L which intrinsically enhances T cell survival. CD28-mediated co-stimulatory signals are responsible for Bcl-x_L upregulation via activation of the NF-κB pathway. Blockade of either co-stimulatory signals or the NF-κB pathway results in tolerance by induction of T cell apoptosis in WT mice, but not in mice expressing a *Bcl-x_L* transgene. These results demonstrate the critical role of passive T cell death in tolerance induction. It has been proposed that apoptotic reduction of the large pool of alloreactive T cells is required for tolerance induction (Wells et al. 1999). Because PKC-θ is a critical signaling molecule required for T cell activation and survival, the function of PKC-θ in allograft rejection was tested using a cardiac rejection model (Manicassamy et al. 2008). Recombination activating gene 1 $(Rag1)^{-/-}$ mice reconstituted with WT T cells readily rejected fully mismatched cardiac allografts, whereas $Rag1^{-/-}$ mice reconstituted with $PKC\text{-}\theta^{-/-}$ T cells failed to promote rejection, suggesting that PKC-θ is required for T cell-mediated allograft rejection. Since PKC-θ is required for survival of activated T cells (Manicassamy et al. 2006a), the role of PKC-θ-regulated survival in cardiac allograft rejection was tested and demonstrated that transgenic expression of Bcl-x_L in $PKC\text{-}\theta^{-/-}$ T cells was sufficient to restore cardiac allograft rejection (Manicassamy et al. 2008). In contrast to adoptive transfer experiments, intact $PKC\text{-}\theta^{-/-}$ mice displayed a delayed but successful cardiac allograft rejection, suggesting a potential compensatory pathway for PKC-θ function. Finally, subtherapeutic doses of anti-CD154 antibody or Cytotoxic T-Lymphocyte Antigen 4 Immunoglobulin (CTLA4-Ig), which were not sufficient to prevent cardiac allograft rejection in the WT mice, prevented heart rejection in the $PKC\text{-}\theta^{-/-}$ mice. This data strongly indicates that, in combination with other treatments, inhibition of PKC-θ can achieving long-term survival of allografts (Manicassamy et al. 2008; Wang et al. 2009).

Conclusion

PKC-θ controls fundamental processes in T cells that integrate TCR and CD28 signals leading to the activation of transcription factors, NF-κB, AP-1, and NFAT, which are essential for productive T cell activation and differentiation. Because PKC-θ regulates T cell-mediated immune responses in vivo, selective PKC-θ inhibitors are believed to have potential clinical applications in the treatment of autoimmunity and prevention of allograft rejection. However, PKC-θ regulates multiple T cell differentiation processes, and the detailed mechanisms involved

are not yet fully understood. Therefore, additional questions need to be addressed prior to clinical testing of PKC-θ inhibitors, including how PKC-θ mediates multiple signaling pathways, and how inhibition of PKC-θ affects normal T cell function in vivo. It is encouraging to report that many pharmaceutical companies have developed selective PKC-θ inhibitors, and therefore, many PKC-θ-regulated functions can be evaluated using these inhibitors instead of the $PKC\theta^{-/-}$ mice, which have potential developmental caveats. With the availability of PKC-θ inhibitors, it is now possible to test their efficacy in autoimmune diseases and allograft rejection, which in all likelihood will lead to clinical trials of PKC-θ-based treatments for human diseases (Kwon et al. 2010).

Cross-References

- ▶ B7 and CD28 Families
- ▶ Cytotoxic T Lymphocytes
- ▶ NF-κB
- ▶ Nuclear Factor of Activated T Cells (NFAT)
- ▶ PI3K
- ▶ T Cell Memory
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver

References

Altman A, Isakov N, Baier G. Protein kinase Ctheta: a new essential superstar on the T-cell stage. Immunol Today. 2000;21:567–73.

Barouch-Bentov R, Lemmens EE, Hu J, Janssen EM, Droin NM, Song J, Schoenberger SP, Altman A. Protein kinase C-theta is an early survival factor required for differentiation of effector CD8+ T cells. J Immunol. 2005;175:5126–34.

Dustin ML. Modular design of immunological synapses and kinapses. Cold Spring Harb Perspect Biol. 2009;1:a002873.

Kwon MJ, Wang R, Ma J, Sun Z. PKC-theta is a drug target for prevention of T cell-mediated autoimmunity and allograft rejection. Endocr Metab Immune Disord Drug Targets. 2010;10:367–72.

Li Y, Hu J, Vita R, Sun B, Tabata H, Altman A. SPAK kinase is a substrate and target of PKCtheta in T-cell receptor-induced AP-1 activation pathway. EMBO J. 2004;23:1112–22.

Ma J, Ding Y, Fang X, Wang R, Sun Z. Protein kinase C-theta inhibits inducible regulatory T cell differentiation via an AKT-Foxo1/3a-Dependent pathway. J Immunol. 2012; Epub 2012/04/28.

Manicassamy S, Gupta S, Huang Z, Sun Z. Protein kinase C-{theta}-mediated signals enhance CD4+ T cell survival by up-regulating Bcl-xL. J Immunol. 2006a;176:6709–16.

Manicassamy S, Gupta S, Sun Z. Selective function of PKC-theta in T cells. Cell Mol Immunol. 2006b;3:263–70.

Manicassamy S, Yin D, Zhang Z, Molinero LL, Alegre ML, Sun Z. A critical role for protein kinase C-theta-mediated T cell survival in cardiac allograft rejection. J Immunol. 2008;181:513–20.

Marsland BJ, Soos TJ, Spath G, Littman DR, Kopf M. Protein kinase C theta is critical for the development of in vivo T helper (Th)2 cell but not Th1 cell responses. J Exp Med. 2004;200:181–9.

Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. Nature. 1998;395:82–6.

Newton AC. Regulation of protein kinase C. Curr Opin Cell Biol. 1997;9:161–7.

Pfeifhofer C, Kofler K, Gruber T, Tabrizi NG, Lutz C, Maly K, Leitges M, Baier G. Protein kinase C theta affects Ca2+ mobilization and NFAT cell activation in primary mouse T cells. J Exp Med. 2003;197:1525–35.

Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhi L, Annes J, Petrzilka D, Kupfer A, Schwartzberg PL, et al. PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. Nature. 2000;404:402–7.

Tan SL, Zhao J, Bi C, Chen XC, Hepburn DL, Wang J, Sedgwick JD, Chintalacharuvu SR, Na S. Resistance to experimental autoimmune encephalomyelitis and impaired IL-17 production in protein kinase C theta-deficient mice. J Immunol. 2006;176:2872–9.

Wang L, Xiang Z, Ma LL, Chen Z, Gao X, Sun Z, Williams P, Chari RS, Yin DP. Deficiency of protein kinase C-theta facilitates tolerance induction. Transplantation. 2009;87:507–16.

Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. Cell. 1994;76:263–74.

Wells AD, Li XC, Li Y, Walsh MC, Zheng XX, Wu Z, Nunez G, Tang A, Sayegh M, Hancock WW, et al. Requirement for T-cell apoptosis in the induction of peripheral transplantation tolerance. Nat Med. 1999;5:1303–7.

Zanin-Zhorov A, Ding Y, Kumari S, Attur M, Hippen KL, Brown M, Blazar BR, Abramson SB, Lafaille JJ, Dustin ML. Protein kinase C-theta mediates negative feedback on regulatory T cell function. Science. 2010;328:372–6.

Platelets, Atherosclerosis, and Immunity

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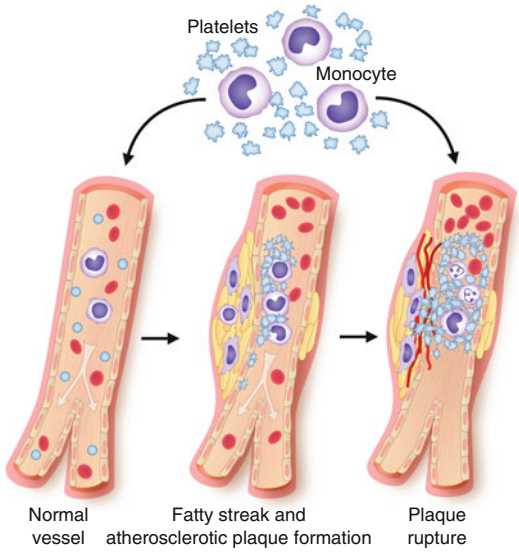
Definition

Platelets are anucleate, disc-shaped, colorless blood cells that are derived from a parent cell type, the megakaryocyte. Platelets have major functions in hemostasis and are the chief hemostatic cells in the blood. In addition to direct hemostatic activities, platelets contribute to activation of plasma coagulation proteins leading to fibrin formation (blood clotting). Platelets also have major inflammatory activities that include innate immune and adaptive immune functions.

There are no commonly used synonyms for platelet in modern medicine and biology. Terms that have been used in the past include Bizzozero's corpuscle, blood disc, blood plate, Deetjen's body, elementary body, elementary particle, Hayem's hemoblast, hemolamella, third corpuscle, Zimmermann's corpuscle, Zimmermann's elementary particle, and Zimmermann's granule. Thrombocyte is a term used for nucleated blood cells that perform the same functions as platelets in nonmammalian vertebrates, whereas mammalian platelets do not have nuclei.

Introduction

Atherosclerosis is a chronic syndrome that usually affects arteries in a discontinuous and segmental fashion. Immune cells and pathways are clearly involved in experimental and clinical atherosclerosis, and it is now accepted that atherosclerosis is a chronic inflammatory disease (Ross 1999; Galkina and Ley 2009; Kaplan 2009). While progressive insidious narrowing of vessels with local limitation of blood flow is a pathologic consequence, a second – and more clinically significant – outcome is ulceration or rupture of atherosclerotic plaques, which results in acute coronary syndromes, stroke, and ischemic peripheral vascular complications (Fig. 1). These acute sequelae are common, characteristically sudden, and often lethal. There is evidence that platelets contribute to both the smoldering progression of atherosclerosis and to the acute, explosive ischemic events (Figs. 1 and 2). In coronary artery plaque rupture triggering myocardial infarction and other acute coronary syndromes, and in carotid plaque ulceration or rupture, which precipitates ischemic stroke, platelets mediate vascular thrombosis (Davi and Patrono 2007; Michelson 2010; Nieswandt et al. 2011) (Figs. 1 and 3). This is consistent with the well-known activities of platelets as chief effector cells of pathologic, as well as physiologic, hemostasis (Michelson 2010; Nieswandt et al. 2011). In addition, however, there is emerging evidence that platelets are key inflammatory and immune effector cells in the initiation and progression of atherosclerosis, which set the stage for atherosclerotic complications. Thus, the activities of platelets in atherosclerosis are not limited to thrombosis and acute vascular occlusion. Their contributions to the chronic inflammation of atherosclerosis are consistent with abundant evidence from studies of their physiologic functions and their contributions to other inflammatory diseases and syndromes that demonstrate that they are major immune effector cells (reviewed in (Vieira-de-Abreu et al. 2012a, b)).

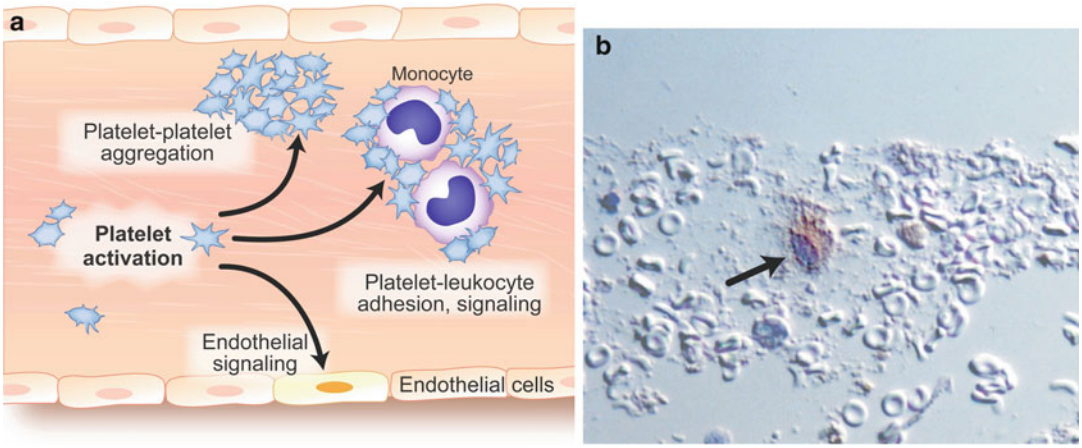


Platelets, Atherosclerosis, and Immunity, Fig. 1 Inflammatory activities of platelets contribute to the evolution of atherosclerotic plaques, which rupture and cause arterial occlusion. Early changes of atherosclerosis involve dysfunction and activation of endothelial cells, altering normal arteries (*left figure*) and initiating formation of "fatty streaks" consisting of monocytes, lipid-laden macrophages (foam cells), T lymphocytes, and extracellular lipids (*middle figure*). Fatty streaks progress to established plaques, which consist of additional leukocytes (monocytes, macrophages, and lymphocytes), smooth muscle cells, complex lipids and lipid crystals, and debris covered by a fibrin cap (Ross 1999). Recent studies utilizing mouse models of atherogenesis indicate that there is substantial leukocyte diversity in established plaques, including the presence of lymphocyte subsets and vascular dendritic cells, and that angiogenesis occurs in the plaques (Galkina and Ley 2009). Platelets are present on the intimal (endothelial) surface at each stage (Ross 1999) and can mediate endothelial activation, leukocyte accumulation and activation, lipid deposition, smooth muscle cell recruitment, angiogenesis, and fibrous responses (Davi and Patrono 2007; Michelson 2010; Nieswandt et al. 2011; Langer et al. 2012; Vieira-de-Abreu et al. 2012a, b). In atherosclerotic plaque rupture (*right figure*), platelets are exposed to collagen, other matrix proteins, lipids and lipid crystals, and leukocytes in the exposed core of the plaque (also see Panel B in Fig. 2). This environment causes intense local platelet activation, precipitating thrombosis (Davi and Patrono 2007; Michelson 2010; Nieswandt et al. 2011). Platelets can release metalloproteinases and locally interact with and signal monocytes, polymorphonuclear leukocytes (PMNs), and macrophages (Langer et al. 2012; Vieira-de-Abreu et al. 2012a, b), events that can amplify plaque instability and rupture. Platelet-monocyte and platelet-PMN aggregates form as a consequence of plaque rupture (Michelson et al. 2001) and can potentially mediate local, regional (i.e., in the coronary circulation), and/or systemic inflammation

Platelets as Immune Effector Cells

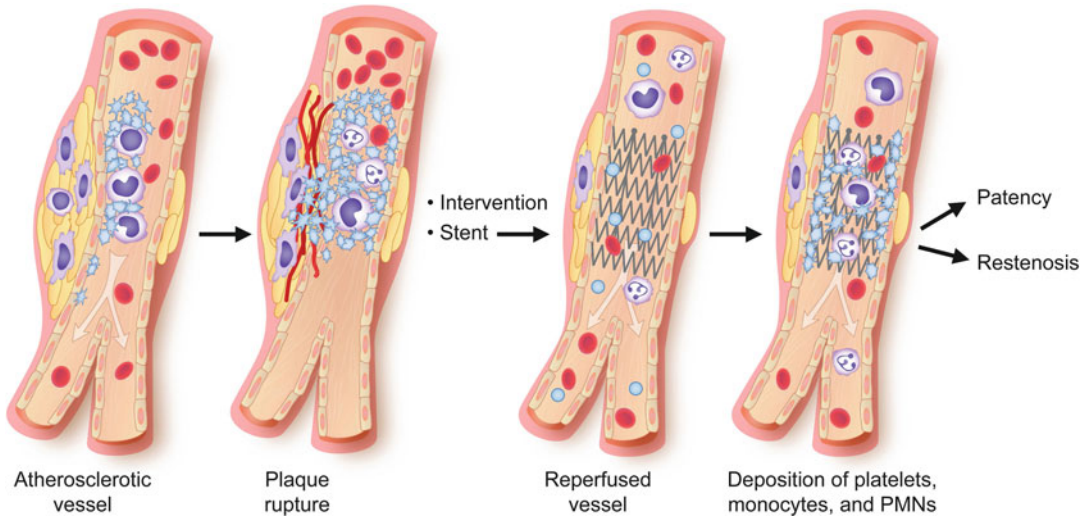
Platelets likely evolved from ancient multifunctional defensive cells with procoagulant, wound sealing, and pathogen capture, containment, and elimination functions. "Modern" platelets are unique, small, anucleate cells that are progeny of polyploid megakaryocytes (Davi and Patrono 2007; Vieira-de-Abreu et al. 2012a). Polyploid megakaryocytes and anucleate platelets are found only in mammals (Michelson 2010), suggesting key cellular specializations driven by evolutionary pressures. Platelets circulate in large numbers under normal conditions and in intact vessels they are preferentially distributed to regions of the blood stream adjacent to endothelial surfaces by rheologic features of the flowing blood and mechanical influences of larger blood cells (Michelson 2010). Platelets are first responders to endothelial injury and have intricate and extensive mechanisms for initiation and propagation of hemostasis at sites of injury and for clot remodeling (Davi and Patrono 2007; Michelson 2010; Nieswandt et al. 2011; Vieira-de-Abreu et al. 2012a). Thus, primary hemostasis is a central function and a chief specialization of platelets. Nevertheless, platelets also have activities that span the continuum from innate to adaptive immune responses and that contribute to complex cellular interactions in acute and chronic inflammation (Vieira-de-Abreu et al. 2012a, b).

Cellular activation is a critical event in the physiologic and pathologic behavior of platelets, dramatically altering their hemostatic and inflammatory phenotypes (Michelson 2010; Vieira-de-Abreu et al. 2012a). Platelets circulate in a resting, quiescent state under basal conditions. In response to activating signals, they rapidly undergo functional alterations that mediate primary hemostasis and also inflammation and immune responses (Davi and Patrono 2007; Michelson 2010; Nieswandt et al. 2011; Vieira-de-Abreu et al. 2012a). A classic and intensely studied general mechanism of platelet activation involves binding of specific ligands to G protein-coupled receptors on the platelet plasma membrane. These include receptors



Platelets, Atherosclerosis, and Immunity, Fig. 2 Platelets have an extensive repertoire of mechanisms that can drive atherogenesis and mediate inflammation in response to plaque rupture or ulceration. *Left panel*. Platelets are activated by agonists generated in atherosclerotic risk conditions and in evolving atherosclerosis (Davi and Patrono 2007; Michelson 2010; Langer et al. 2012). Platelet activation can induce their adhesion to endothelial surfaces, aggregation, release of preformed proatherogenic chemokines and cytokines (PF4, RANTES, CD40L, others), posttranscriptional synthesis of IL-1β, tissue factor, and other inflammatory gene products, and adhesive interactions with monocytes, lymphocytes, and other leukocyte subsets by mechanisms that include binding of P-selectin on activated platelets to PSGL-1 on leukocytes. Adhesive interactions of activated platelets with leukocytes facilitate their deposition on local endothelial surfaces and transmigration into plaques and also cause the formation of locally deposited and circulating platelet-leukocyte aggregates. Signaling of monocytes by platelets in aggregates induces the transcriptional and posttranscriptional expression of a variety of proinflammatory proteins and generation of other mediators (eicosanoids, oxygen radicals). Platelets can also deliver signals to endothelial cells that activate them and alter their gene expression profiles and synthetic functions, potentially amplifying very early endothelial activation events. These and other features of the innate and adaptive immune repertoire of activated platelets may be key pathogenetic features in atherogenesis (Davi and Patrono 2007; Langer et al. 2012). *Right panel*. Tissue from an ulcerated carotid artery plaque with a central macrophage (*arrow*) surrounded by adherent platelets and accumulated erythrocytes is shown. Platelet-platelet and platelet-leukocyte interactions mediate inflammatory responses (reviewed in (Vieira-de-Abreu et al. 2012a, b)) that may be important determinants in the acute, subacute, and long-term outcomes of plaque rupture (Davi and Patrono 2007; Langer et al. 2012) (Panel is reprinted from Weyrich AS, Lindemann S, Zimmerman GA. The evolving role of platelets in inflammation. J Thromb Haemost. 2003;1:1897–905. with permission)

that recognize the hemostatic and inflammatory protease thrombin (protease-activated receptors), purinoceptors that are activated by ADP released from platelet dense granules, the receptor for the endogenously synthesized eicosanoid thromboxane A_2, and other G protein-coupled receptors that recognize diverse ligands and agonists including 5-hydroxytryptamine, platelet-activating factor, and certain prostaglandins (Davi and Patrono 2007; Michelson 2010; Vieira-de-Abreu et al. 2012a). Platelet G protein-coupled receptor pathways are targets for molecular therapy in cardiovascular diseases (Michelson 2010). It has recently been recognized that platelets have toll-like receptors (TLRs), a major class of receptors that recognize microbial products and other exogenous and endogenous ligands. TLRs are critical in immune signaling, and expression of functional TLRs by human and murine platelets is further evidence for inflammatory and immune specializations of these cells (reviewed in (Vieira-de-Abreu et al. 2012a)). TLR signaling is a component or the pathophysiology of atherosclerosis based on studies in mice (Galkina and Ley 2009). Platelets also have a number of other classes of receptors, providing a diverse signaling repertoire. These include "immunoreceptors" such as classic Fc receptors and a unique platelet adhesive and signaling factor, glycoprotein VI (GPVI)



Platelets, Atherosclerosis, and Immunity, Fig. 3 Platelets are rapidly deposited in plaque rupture and vascular stenting and mediate leukocyte accumulation and other inflammatory responses. Evolution of atherosclerotic plaques frequently leads to plaque ulceration or rupture (*left figures*). Platelets are rapidly deposited at sites of atherosclerotic plaque ulceration or rupture and mediate local vascular thrombosis. Platelet activation and local cellular interactions also induce inflammation, in addition to pathologic hemostasis (see Fig. 1, the *right panel* of Fig. 2, and text). Vascular intervention, including stent placement, is a commonly utilized approach together with antithrombotic and other pharmacologic management. Activated platelets are rapidly deposited at sites of angioplasty and intravascular stent deployment and mediate monocyte and PMN deposition in these regions (Palmerini et al. 2002; Welt and Rogers 2002) (*right figure*). Activated platelets and myeloid leukocytes may be key cellular determinants of restenosis rather than long-term patency after stent placement, based on experimental studies (Welt and Rogers 2002). (Reprinted from Rondina et al. 2013 with permission)

(Nieswandt et al. 2011; Vieira-de-Abreu et al. 2012a). Outside-in activating signals delivered by platelet receptors are transmitted to transduction pathways that are linked to functional responses (Davi and Patrono 2007; Michelson 2010; Nieswandt et al. 2011; Vieira-de-Abreu et al. 2012a). Both hemostatic and inflammatory/immune responses of activated platelets are frequently induced by the same receptors and use common signal transduction cascades. Although many functional responses of activated platelets are rapidly induced, depending on the specific receptor system, some of the functional alterations are also sustained over hours based on in vitro studies. This contrasts with the traditional idea that all important functional responses of activated platelets are over in a few minutes.

Responses of activated platelets include (reviewed in (Davi and Patrono 2007; Michelson 2010; Nieswandt et al. 2011; Vieira-de-Abreu et al. 2012a)) amplified adhesion to exposed collagen and subendothelial matrix; inside-out signaling of integrin $\alpha_{II}\beta_3$ (GPIIb/IIIa) and other surface integrins; binding of fibrinogen and fibrin; aggregation; presentation of a procoagulant surface; degranulation with surface translocation of P-selectin and secretion of preformed chemokines, cytokines, and antimicrobial peptides; cleavage and release of surface CD40 ligand (CD40L; CD154); and intercellular interactions and signaling of endothelial cells, leukocytes, and – in some cases – extravascular cells. Many of these responses have been recognized and studied for decades. In addition, however, recent discoveries have revealed "new biology" of platelets, including the ability to undergo cellular fission and the capacity to alter the resting platelet proteome by activation-dependent processing and translation of RNA species that make up a complex and extensive transcriptome in these cells (reviewed in (Vieira-de-Abreu et al. 2012a, b)). In parallel,

it is now recognized that platelets participate in adaptive immune responses in addition to being important innate immune effector cells and that they have diverse activities in acute and chronic infection and inflammatory diseases (reviewed in (Vieira-de-Abreu et al. 2012a, b)). Some or all of these activities may operate in human subjects with atherosclerosis and may contribute to the development of intimal plaques and/or to the complications of plaque rupture (Fig. 1).

The capacity to signal other cells is key in the inflammatory and immune activities of platelets, including their contributions to atherogenesis and its pathologic sequelae (Langer et al. 2012; Vieira-de-Abreu et al. 2012a). The signaling repertoire of activated platelets is extensive both in terms of the diversity of molecular signaling factors (see next section) and modes of intercellular signaling. The latter include juxtacrine signaling by factors anchored in the platelet plasma membrane, release of soluble paracrine signaling molecules, and release of microvesicles (microparticles) that transport and present signaling factors to "target" cells (reviewed in (Vieira-de-Abreu et al. 2012a)).

Platelets in the Initiation of Atherosclerosis and Development of Atherosclerotic Plaques: Pathogenesis and Experimental Observations

The precise events that initiate atherosclerosis are not yet known, but platelets may be key effector cells early in the process. Mechanisms traditionally proposed for atherogenesis include endothelial injury and/or dysfunction, and inappropriate plasma levels and dysregulated uptake of cholesterol and oxidatively modified phospholipids (Ross 1999; Galkina and Ley 2009; Kaplan 2009). There is experimental and clinical evidence that these are critical and, likely, interacting pathogenetic events that then drive progressive inflammation in regions of the arterial endothelium that are destined for plaque involvement, dictated in part by hemodynamic forces (Ross 1999; Galkina and Ley 2009). Additional mechanisms have been proposed, including local vascular infection and/or pathologic endothelial responses to a systemic pathogen burden, reactivity to self-antigens, and autoimmunity (Ross 1999; Galkina and Ley 2009; Kaplan 2009). Because platelets are immediate responders to vascular injury, and because inflammation is a fundamental biologic response to metabolic and mechanical as well as traumatic tissue damage, it is likely that platelets are involved early in atherogenesis and that the inflammatory and immune repertoire of platelets contributes to the initiation and progression of atherogenesis. Platelets respond directly to oxidatively modified lipids and oxidized low-density lipoprotein (LDL) (Marathe et al. 2002; Podrez et al. 2007) and to a variety of agonists generated as a consequence of vascular injury (Davi and Patrono 2007; Michelson 2010). Reactive oxygen species may link arachidonate conversion and isoprostane formation, platelet activation, and leukocyte accumulation in conditions that predispose to atherosclerosis, including diabetes, obesity, hyperhomocystemia, and smoking (Davi and Patrono 2007). These observations suggest that there may be a plethora of early signals that induce platelet activation in atherosclerosis.

Platelet adhesion to endothelium in areas of atherosclerotic involvement and to the surfaces of early fatty streaks and established plaques (Fig. 1) is said to be ubiquitous in experimental animals and humans (Ross 1999). Studies in mice, although yet of uncertain relevance to human atherogenesis (Rader 2012), support this conclusion and suggest that platelet accumulation on atherosclerotic endothelium has pathogenetic relevance. Some of the key observations include the following (reviewed in (Davi and Patrono 2007; Langer et al. 2012)): Platelets were found to adhere to the endothelium in arteries of mice genetically deficient in apolipoprotein E (*Apoe*$^{-/-}$), a commonly used model, before atherosclerotic lesions were detectable. Genetic deletion of the α_{IIb} subunit of integrin $\alpha_{IIb}\beta_3$, which is unique to platelets and megakaryocytes and is critical in platelet aggregation, adhesion, and outside-in signaling, reduced atherosclerotic involvement of the aorta and carotid arteries in

Apoe$^{-/-}$ animals. Bone marrow transplant experiments in mice deficient in P-selectin indicated that platelet P-selectin is involved in atherosclerotic lesion development. In addition, circulating activated platelets contributed to atherogenesis in *Apoe*$^{-/-}$ mice by secreting proinflammatory chemokines (see below) and by forming platelet-monocyte aggregates and promoting leukocyte accumulation on endothelial surfaces in a P-selectin-dependent manner. These experimental findings are consistent with observations that platelets and platelet products are found with monocytes, lipid-laden macrophages, and lymphocytes on, and/or in, fatty streaks and atherosclerotic plaques (Fig. 1). In addition, inhibition of synthesis of thromboxane A_2 (TXA_2), a major metabolite of arachidonic acid in activated platelets, TXA_2 receptor blockade, or genetic deletion of the TXA_2 receptor retarded atherosclerotic development in mouse models. In recent studies, inhibition of GPVI, which is expressed only by platelets, reduced atherosclerotic progression in rodent models.

The functional repertoire of activated platelets provides a variety of inflammatory and immune mechanisms that may be involved in their contributions to atherosclerotic initiation and progression (Fig. 2). Intercellular signaling is one of these (reviewed in (Vieira-de-Abreu et al. 2012a)) and is critical in activities of platelets in other inflammatory and immune syndromes and diseases (reviewed in (Vieira-de-Abreu et al. 2012b)). P-selectin on the plasma membranes of activated platelets engages a ligand, P-selectin glycoprotein (PSGL-1), on monocytes, neutrophils, and lymphocyte subsets, binding the cells together to form aggregates (Fig. 2). In human studies, formation of platelet-monocyte aggregates and their detection in circulating blood have been reported in conditions that predispose to atherosclerosis, including diabetes and smoking (Vieira-de-Abreu et al. 2012b), and in established atherosclerosis and acute coronary syndromes (Michelson et al. 2001; Michelson 2010). Platelet-monocyte aggregates also form in baboons (Michelson et al. 2001) and in atherosclerosis-prone mice (Huo et al. 2003). Interactions of platelets with leukocytes in this manner can facilitate adhesion of specific leukocyte classes – including monocytes, which are ubiquitously present at all stages of atherogenesis (Ross 1999) – to areas of activated endothelium and promote transmigration of the white blood cells to subendothelial domains (reviewed in (Vieira-de-Abreu et al. 2012a)). These are key features in fatty streak formation and plaque evolution (Ross 1999; Galkina and Ley 2009). Engagement of PSGL-1 on monocytes by P-selectin on activated platelets also delivers outside-in signals to the leukocyte, alone or in combination with additional paracrine and adhesive signals from the platelet or, in some cases, autocrine signals from the monocyte (Dixon et al. 2006; Vieira-de-Abreu et al. 2012a). These molecular signaling cues induce functional alterations in monocytes, including new or altered expression of inflammatory gene products that have demonstrated, or potential, activities in atherosclerosis (reviewed in (Vieira-de-Abreu et al. 2012a)). Binding of platelets to monocytes by interaction of P-selectin with PSGL-1 also influences cell fate determination, delivering signals that induce differentiation of monocytes to macrophages or, in contrast, to dendritic cells (reviewed in (Vieira-de-Abreu et al. 2012a)). This may be important because monocyte-derived lipid-laden macrophages and other macrophage subsets are present throughout the evolution of atherosclerotic lesions and are key cellular components of established plaques (Ross 1999; Galkina and Ley 2009), and because there is recent evidence for vascular dendritic cell networks in the arterial intima of humans and rodents (Galkina and Ley 2009; Langer et al. 2012). The precise activities of dendritic cells in atherogenesis are not yet clear (Galkina and Ley 2009; Langer et al. 2012). Activated platelets also directly interact with macrophages, dendritic cells, and lymphocyte subclasses (Vieira-de-Abreu et al. 2012a). These and other observations indicate that activated platelets are intermediaries of information transfer between key cells that can initiate and drive complex inflammatory and immune pathways in atherosclerosis, including cellular dialogues that link innate and adaptive immune responses. Activated platelets also

deliver molecular signals that trigger major inflammatory responses of endothelial cells (Langer et al. 2012; Vieira-de-Abreu et al. 2012a, b) (see Fig. 2 and below).

In addition to adhesion-dependent signaling of leukocytes mediated by P-selectin and PSGL-1, activated platelets deliver paracrine or juxtacrine chemokine and cytokine signals that can trigger proinflammatory events in atherogenesis and fan the fire of vascular inflammation (Davi and Patrono 2007; Galkina and Ley 2009; Langer et al. 2012). Chemokines and cytokines are components of a complex platelet secretome that includes more than 300 identified factors (Vieira-de-Abreu et al. 2012a). Activated human and murine platelets release chemokines of both the CXC (characterized by a single amino acid residue between the first two cysteine residues) and CC (characterized by the first two cysteine residues adjacent to one another) classes. Platelet factor 4 (PF4, CXCL4) and regulated upon activation, normal T expressed, and presumably secreted (RANTES; CCL5), which are constitutively present in platelet granules and are released on activation (Vieira-de-Abreu et al. 2012a), may be key mediators of atherogenesis based on experimental observations. PF4 activates monocytes and induces their differentiation to macrophages, inhibits LDL catabolism and promotes uptake of oxidized LDL by macrophages, and induces binding of oxidized LDL to other cells of the vascular wall (reviewed in (Langer et al. 2012)). Activated platelets are reported to deposit heterodimers of PF4 and RANTES on endothelium, triggering monocyte arrest (Huo et al. 2003; Koenen et al. 2009). RANTES is a pleiotropic mediator of inflammation (reviewed in (Vieira-de-Abreu et al. 2012a)) and has the potential to drive multiple cellular events in atherosclerotic inflammation (reviewed in (Galkina and Ley 2009; Koenen et al. 2009; Langer et al. 2012)). In $Apoe^{-/-}$ mice, inhibitors that disrupted PF4-RANTES interactions reduced monocyte recruitment and atherosclerotic involvement of the aorta (Koenen et al. 2009). Targeted deletion of CCR5, a chemokine receptor that recognizes RANTES, yielded mixed results in $Apoe^{-/-}$ mice (reviewed in (Galkina and Ley 2009)).

Together with chemokines, platelet cytokines also deliver signals that can drive atherogenesis. Interleukin-1β (IL-1β), a pleiotropic inflammatory cytokine and immune modulator, is released by activated platelets associated with microparticles and in soluble form; in addition, IL-1β mediates juxtacrine signaling while retained on the platelet plasma membrane and is also deposited in model thrombi, suggesting that clots serve as an intravascular cytokine reservoir (reviewed in (Vieira-de-Abreu et al. 2012a)). Activated human and mouse platelets synthesize IL-1β by a mechanism that involves regulated splicing of the *IL-1β* pre-mRNA and translation of the mature, processed transcript (reviewed in (Vieira-de-Abreu et al. 2012b)). In addition to IL-1β, other protein products including tissue factor (Schwertz et al. 2006) – which has prothrombotic activities in atherosclerosis and its complications – are synthesized by activated platelets in this fashion (Davi and Patrono 2007; Vieira-de-Abreu et al. 2012a). IL-1β synthesized by activated platelets induces inflammatory responses by monocytes, endothelial cells, and other cell types both in vitro and in vivo (reviewed in (Vieira-de-Abreu et al. 2012a, b)). IL-1β has been linked to atherogenesis by a variety of observations, including experiments indicating that lack of IL-1β decreases atherosclerotic burden in the $Apoe^{-/-}$ murine model and more recent studies in which transfer of bone marrow genetically deficient in IL-1β or in the NLRP3 inflammasome – which converts pro-IL-1β to the soluble, active cytokine – reduced atherosclerotic involvement in LDL receptor-deficient mice (reviewed in (Galkina and Ley 2009; Rader 2012)). A clinical trial is currently under way to test the possibility that inhibition of IL-1β with a humanized monoclonal antibody will reduce major cardiovascular events in subjects with preexisting atherosclerotic disease (Rader 2012). Recent murine studies suggest that IL-1 signaling may have complicated effects on atherosclerotic vessels, however, and can mediate both pathologic and compensatory alterations (Alexander et al. 2012; Rader 2012). This provides a cautionary note to be considered in interventional clinical trials and illustrates the

key point that inflammation is fundamental to protection of the host and to tissue repair and that beneficial inflammatory activities may occur in parallel with pathologic inflammatory events. Thus, anti-inflammatory interventions in atherosclerosis and other inflammatory conditions may have unintended negative consequences (Rader 2012). The cellular sources of IL-1β in clinical and experimental atherosclerosis, and the extent to which platelet-derived IL-1β contributes, are unknown. Activated platelets also induce IL-1β synthesis by monocytes (Dixon et al. 2006), suggesting a mechanism by which effects of platelet-derived IL-1β could be amplified in atherosclerosis and other disorders.

CD40 ligand (CD40L; CD154) is rapidly translocated from intracellular stores to the surfaces of activated platelets. Cleavage of CD40L yields a soluble, biologically active, cytokine fragment; platelets are the principal source of plasma CD40L (Davi and Patrono 2007; Vieira-de-Abreu et al. 2012a). CD40L and its receptor, CD40 – which is also found on platelets – are immune modulators of the tumor necrosis factor (TNF) and TNF receptor families that mediate intricate interactions between lymphocytes, myeloid leukocytes, and endothelial cells. Early studies indicated that genetic deletion or antibody inhibition of CD40L reduces atherosclerotic involvement in *Apoe*$^{-/-}$ mice (Galkina and Ley 2009; Langer et al. 2012). In addition, in vitro experiments provided mechanistic correlates, including evidence that activation of human endothelial cells occurs in response to juxtacrine signaling mediated by platelet-associated CD40L (reviewed in (Langer et al. 2012)). More recently, injection of *CD40L*$^{+/+}$ platelets into *Apoe*$^{-/-}$ mice was reported to induce platelet-leukocyte aggregate formation and platelet and leukocyte accumulation at endothelial surfaces and to accelerate atherosclerotic plaque development. In contrast, these events were inhibited when *CD40L*$^{-/-}$ platelets were injected, and *CD40L*$^{-/-}$ platelets did not induce atherosclerotic lesions. Platelet CD40L also influenced levels of regulatory T lymphocytes in the blood and spleen in this study (Lievens et al. 2010). These observations indicate that the CD40L-CD40 axis, like the IL-1 signaling system and the chemokines PF4 and RANTES, may be a key component of initiating and/or amplifying cascades in atherosclerotic inflammation (Davi and Patrono 2007; Langer et al. 2012). Targeted deletion of CD40, the ligand for CD40L, in LDL receptor-null mice did not reduce atherosclerotic lesion formation, suggesting alternative signaling pathways (Galkina and Ley 2009).

Platelets as Immune Effector Cells in Atherosclerosis: Clinical Correlates

Immunohistochemical analysis of human atherosclerotic vascular tissues indicates that the deposition of PF4 is associated with lesion severity and with symptomatic atherosclerosis, suggesting that persistent platelet activation contributes to the evolution of atherosclerotic vasculopathy (Langer et al. 2012). This further supports the idea that platelets are present early and continuously at sites of developing fatty streaks and maturing atherosclerotic plaques (see above and Figs. 1 and 2) (Ross 1999). In addition, several other platelet chemokines and inflammatory mediators are reported to be present in atherosclerotic plaques (Davi and Patrono 2007). Further clinical evidence also suggests that platelets are effectors in clinical atherosclerosis. This includes the following (reviewed in (Davi and Patrono 2007)): High plasma levels of CD40L were reported to be a marker of risk for vascular events in a prospective study of healthy women. Persistent platelet activation, detected by increased levels of thromboxane metabolites, has been associated with risk factors that contribute to accelerated atherogenesis in clinical studies. Additionally, risk factors including cigarette smoking, type 2 diabetes, and the combination of hyperinsulinemia and hyperglycemia are associated with platelet activation and increased plasma levels of CD40L. In noninvasive studies, platelet activation was associated with increased carotid artery wall thickness, and platelet degranulation was correlated with progressive thickening of the intima-media region of the common

carotid artery in patients with type 2 diabetes mellitus. These and other observations (reviewed in (Davi and Patrono 2007)) support the concepts that platelet activation is a central pathogenetic mechanism in clinical atherosclerosis and that the inflammatory and immune effector activities of platelets are induced in conditions that increase risk for atherosclerosis and its complications. These clinical correlates are also consistent with evidence from experimental inquiries that link activated platelets to atherogenesis, as outlined above (reviewed in (Langer et al. 2012)). New clinical and experimental studies examining the activities of platelets in obesity, diabetes, and the metabolic syndrome are in progress and may yield additional mechanistic insights regarding contributions of platelets to atherosclerotic risk imposed by these conditions.

Platelets and Inflammation in Acute Coronary Syndromes and Vascular Intervention

As noted previously, platelets are critical in vascular thrombosis that accompanies acute coronary syndromes and ischemic cerebrovascular events (Davi and Patrono 2007; Michelson 2010; Nieswandt et al. 2011). Their prothrombotic activities may involve newly recognized responses, including synthesis of tissue factor (Schwertz et al. 2006; Davi and Patrono 2007; Vieira-de-Abreu et al. 2012a), and more traditionally studied (Ross 1999; Michelson 2010; Nieswandt et al. 2011) procoagulant mechanisms. Platelet activation and adhesion pathways are targets for molecular therapies aimed at reducing or preventing thrombotic complications in these cardiovascular sequelae of atherosclerosis (reviewed in (Michelson 2010)). Inflammation – in addition to thrombosis – is also a concomitant of acute coronary syndromes (Figs. 1 and 3) (Davi and Patrono 2007), and ischemic stroke is suggested to be a "thrombo-inflammatory" disorder (Nieswandt et al. 2011). Platelets are intensely activated in the regions of ruptured or ulcerated atherosclerotic plaques (Figs. 1–3), and many of their inflammatory and immune effector mechanisms appear to be triggered in this context (reviewed in (Langer et al. 2012)). For example, platelet-monocyte and platelet-PMN aggregates form and circulate in patients with acute myocardial infarction (Michelson et al. 2001). Circulating platelet-monocyte aggregates are a particularly sensitive index of in vivo platelet activation in acute coronary events (Michelson et al. 2001) and – potentially – in other inflammatory syndromes (Vieira-de-Abreu et al. 2012b). "Antiplatelet" therapies for acute atherosclerotic cardiovascular syndromes (Michelson 2010) may interrupt inflammatory activities of platelets, in addition to abrogating their thrombotic responses, and may blunt chronic inflammation when they are administered for the long term after being initiated during treatment for acute atherosclerotic complications (Fig. 1).

Vascular intervention, including angioplasty and placement of intravascular stents, is a common approach to improve flow through critical areas of involvement in atherosclerotic coronary arteries (Fig. 3). Intravascular stents are also being evaluated in atherosclerotic cerebrovascular disease. Vascular interventions can yield prolonged patency, but can be complicated by acute periprocedural or delayed thrombosis. Furthermore, the therapeutic outcome is often impaired by restenosis of the vessel in the stent region (Fig. 3). Rapid platelet deposition and accumulation of polymorphonuclear leukocytes (PMNs) and monocytes are acute local responses to the iatrogenic vascular trauma of stent placement in humans and experimental animals (Palmerini et al. 2002; Welt and Rogers 2002). Platelet-PMN and platelet-monocyte aggregates also form and circulate after percutaneous coronary intervention (Michelson et al. 2001). Later (days to weeks), macrophages accumulate in the stented region and are observed around the stent scaffold (Welt and Rogers 2002). There is experimental evidence that platelets recruit leukocytes to the stent milieu (Palmerini et al. 2002) and that leukocyte accumulation is causally related to neointimal hyperplasia and restenosis (Welt and Rogers 2002). In addition to promoting leukocyte

accumulation, a variety of proinflammatory activities of platelets (Langer et al. 2012; Vieira-de-Abreu et al. 2012a) may contribute to dysregulated repair of the vascular surface and to excessive neointima formation that lead to stent restenosis. Platelet inhibitory therapies can alter acute cellular events in the stented vessel (Palmerini et al. 2002), and it is possible that new or combination approaches (Michelson 2010) may improve long-term outcomes by interrupting proinflammatory activities of platelets in this context. Platelets also have signaling pathways that are altered or interrupted by pharmacologic agents delivered by "drug-eluting" stents including the mammalian target of rapamycin (mTOR) cascade (Vieira-de-Abreu et al. 2012a), which is inhibited by sirolimus (rapamycin) – one of the drugs used to impregnate stents for local delivery in hopes of reducing restenosis.

Chronic Immune and Inflammatory Disorders, Atherosclerosis, and Platelets

The risk of atherosclerotic complications is increased in several chronic immune diseases and syndromes, based on observational, epidemiologic, and prospective controlled investigations. Two examples are rheumatoid arthritis and systemic lupus erythematosus (SLE). Premature atherosclerotic cardiovascular disease develops in a significant number of patients with these diseases (reviewed in (Galkina and Ley 2009; Kaplan 2009)). Activation of circulating platelets occurs in both syndromes, and there is evolving clinical and experimental evidence indicating that platelets are inflammatory effector cells in the extravascular manifestations (articular, renal, other) of rheumatoid arthritis and SLE (reviewed in (Vieira-de-Abreu et al. 2012b)). Thus, platelets may also be central to the amplified and accelerated progression of chronic atherosclerotic vascular inflammation in these and other systemic immune syndromes. Molecular mechanisms that are unique to each inflammatory/immune disorder may converge to trigger and sustain platelet activation (Vieira-de-Abreu et al. 2012b). Therapeutic strategies that include disease-modifying drugs specific to the chronic syndrome (Kaplan 2009) together with mechanistically targeted platelet interventions (Michelson 2010) may ultimately be indentified and used to favorably alter the natural history of atherosclerotic vasculopathy in these disorders.

Cross-References

- ▶ Atherosclerosis and Cytokines
- ▶ CD40
- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Dendritic Cells in Atherosclerosis
- ▶ Lymphocytes in Atherosclerosis
- ▶ Macrophages, Oxidative Stress, and Atherosclerosis
- ▶ Mammalian Target of Rapamycin (mTOR)
- ▶ Systemic Autoimmune Disease and Premature Atherosclerosis

References

Alexander MR, Moehle CW, Johnson JL, Yang Z, Lee JK, Jackson CL, et al. Genetic inactivation of IL-1 signaling enhances atherosclerotic plaque instability and reduces outward vessel remodeling in advanced atherosclerosis in mice. J Clin Invest. 2012;122(1):70–9.

Davi G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med. 2007;357(24):2482–94.

Dixon DA, Tolley ND, Bemis-Standoli K, Martinez ML, Weyrich AS, Morrow JD, et al. Expression of COX-2 in platelet-monocyte interactions occurs via combinatorial regulation involving adhesion and cytokine signaling. J Clin Invest. 2006;116(10):2727–38.

Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. Annu Rev Immunol. 2009;27:165–97.

Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med. 2003;9(1):61–7.

Kaplan MJ. Management of cardiovascular disease risk in chronic inflammatory disorders. Nat Rev Rheumatol. 2009;5(4):208–17.

Koenen RR, von Hundelshausen P, Nesmelova IV, Zernecke A, Liehn EA, Sarabi A, et al. Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. Nat Med. 2009;15(1):97–103.

Langer H, Geisler T, Gawaz M. Atherothrombosis and coronary artery disease. In: Michelson AD, editor. Platelets. 3rd ed. Oxford: Elsevier/Academic Press; 2012.
Lievens D, Zernecke A, Seijkens T, Soehnlein O, Beckers L, Munnix IC, et al. Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. Blood. 2010;116(20):4317–27.
Marathe GK, Zimmerman GA, Prescott SM, McIntyre TM. Activation of vascular cells by PAF-like lipids in oxidized LDL. Vascul Pharmacol. 2002;38(4):193–200.
Michelson AD. Antiplatelet therapies for the treatment of cardiovascular disease. Nat Rev Drug Discov. 2010;9(2):154–69.
Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation. 2001;104(13):1533–7.
Nieswandt B, Pleines I, Bender M. Platelet adhesion and activation mechanisms in arterial thrombosis and ischaemic stroke. J Thromb Haemost: JTH. 2011;9(Suppl 1):92–104.
Palmerini T, Nedelman MA, Scudder LE, Nakada MT, Jordan RE, Smyth S, et al. Effects of abciximab on the acute pathology of blood vessels after arterial stenting in nonhuman primates. J Am Coll Cardiol. 2002;40(2):360–6.
Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, Valiyaveettil M, et al. Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. Nat Med. 2007;13(9):1086–95.
Rader DJ. IL-1 and atherosclerosis: a murine twist to an evolving human story. J Clin Invest. 2012;122(1):27–30.
Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. Circulation Research 2013;112:1506–1519.
Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med. 1999;340(2):115–26.
Schwertz H, Tolley N, Foulks J, Denis M, Risenmay B, Buerke M, et al. Signal-dependent splicing of tissue factor pre-mRNA modulates the thrombogenecity of human platelets. JEM. 2006;203(11):2433–40.
Vieira-de-Abreu A, Campbell RA, Weyrich AS, Zimmerman GA. Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. Semin Immunopathol. 2012a;34:5–30.
Vieira-de-Abreu A, Rondina MT, Weyrich AS, Zimmerman GA. Inflammation. In: Michelson AD, editor. Platelets. 3rd ed. Oxford: Elsevier/Academic Press; 2013;733–766.
Welt FG, Rogers C. Inflammation and restenosis in the stent era. Arterioscler Thromb Vasc Biol. 2002;22(11):1769–76.

Polyarteritis Nodosa

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Definition

A systemic inflammatory disease characterized by necrotizing medium-vessel vasculitis.

History

Polyarteritis nodosa (PAN) was the first vasculitis to be described in the medical literature (Kussmaul and Maier 1866). While the initial descriptions of this illness included clinical manifestations and consequences of necrotizing small- and medium-vessel inflammation, microscopic polyangiitis was described as an independent disease in 1949, and the Chapel Hill Consensus Conference defined PAN strictly as a medium-vessel vasculitis (Jennette et al. 1994).

Epidemiology

The annual incidence of PAN has been reported to be 9.0 per million in Olmsted County, Minnesota (Kurland et al. 1984). Overall, PAN is a rare illness when carefully defined, and successful immunization against hepatitis B has led to a decline in cases associated with this infection. It tends to affect both genders equally and can involve patients of all ages.

Etiology

The etiology of PAN is unknown. It may occur as a primary (idiopathic) process or secondary to

other diseases such as viral infections (hepatitis B, hepatitis C, Human Immunodeficiency Virus infection), other systemic autoimmune or autoinflammatory diseases (such as Familial Mediterranean Fever), or malignancy (such as hairy cell leukemia) Guillevin et al. 2008.

Pathogenesis

PAN is characterized by segmental necrotizing inflammation of arteries with infiltration of neutrophils and monocytes, often with leukocytoclasis, with or without thrombosis (Jennette et al. 2002). The pathogenesis of PAN is incompletely understood, but the formation of immune complexes (composed of antigen, immunoglobulin, and complement) has been implicated with resultant immune activation and vascular inflammation and damage. Although this has been well documented in some models of the disease, it has been inconsistently demonstrated in clinical studies. The deposition of immune complexes at arterial branch points (probably for mechanical reasons related to blood flow) is likely responsible for the location of arterial lesions in PAN. Fibrinoid necrosis is a nonspecific pattern of acute necrotizing injury resulting from many forms of necrotizing vasculitis characterized by accumulation of plasma proteins, including coagulation factors that are converted to fibrin, at sites of tissue destruction. Over time, fibrinoid necrosis is replaced by collagenous scar. Necrotizing inflammation resulting in extensive destruction of the vessel wall and perivascular tissue can have two consequences: aneurysms or pseudoaneurysms from weakening of the vessel wall and/or vascular occlusion with downstream ischemia. Focal activation of neutrophils and monocytes at the interface of blood and artery appears to be an early pathogenic event resulting in leukocyte activation and transmural infiltration of the artery wall and necrotizing injury. Variations in host immune responses undoubtedly play a role in the initiation and maintenance of vascular injury.

Clinical Features

PAN may remain confined to the skin (cutaneous PAN) or involve medium-sized blood vessels in multiple visceral organs. PAN tends to be a systemic illness. In a recent study of patients with PAN (Pagnoux et al. 2010), general symptoms (fever, weight loss, arthralgia, myalgia) were reported in 93.1 %, neurologic manifestations (central and peripheral nervous system) in 79 %, skin involvement (nodules, ulcers, gangrene, livedo reticularis) in 49.7 %, and abdominal pain and hypertension in approximately 35 % each. Approximately 70 % of patients in this study had histologically proven PAN. Hypertension in patients with PAN can be a consequence of renal artery involvement or glucocorticoid therapy. Other organ involvements can include cardiac and gastrointestinal (mesenteric ischemia, hemorrhage, cholecystitis, and intestinal perforation). Patients with HBV-related PAN were reported to have a higher frequency of peripheral neuropathy, abdominal pain, cardiomyopathy, orchitis, and hypertension compared to patients with idiopathic PAN. Orchitis occurs in approximately 25 % patients with PAN; this finding should lead to a suspicion of PAN in the appropriate clinical context. In contrast to vasculitides involving smaller vessels, PAN does not typically cause glomerulonephritis, palpable purpura, and/or alveolar hemorrhage from pulmonary capillaritis. For unclear reasons, pulmonary involvement does not occur in patients with this illness.

Diagnosis

The diagnosis of PAN is based on a compatible clinical presentation accompanied by laboratory test results and/or imaging studies. The clinical presentation of systemic inflammation is often accompanied by concordant laboratory tests (normochromic normocytic anemia, leukocytosis, thrombocytosis, low serum albumin, and elevated erythrocyte sedimentation rate and C-reactive protein). Liver enzymes may be abnormal in the

setting of active viral hepatitis. Clinical features should guide advanced workup for PAN, and invasive testing of asymptomatic areas is not recommended. In patients with abdominal pain, or new-onset hypertension, a conventional dye arteriogram of the mesenteric and renal arterial circulation may reveal microaneurysms. Other vascular imaging modalities such as CT angiography or magnetic resonance angiography are not sensitive enough to detect the presence of microaneurysms. The presence of microaneurysms is not pathognomonic of PAN and should be interpreted as evidence supportive of PAN in patients with a compatible clinical picture. Similarly, nerve and muscle biopsy (typically sural nerve and gastrocnemius muscle) can be associated with significant morbidity and should not be performed in patients without clinical peripheral nerve involvement. Since renal involvement in PAN is not from glomerulonephritis, there is no role for a renal biopsy, and this procedure may actually be associated with the risk of hemorrhage from rupture of a renal artery microaneurysm. Patients with PAN typically do not elaborate anti-neutrophil cytoplasmic antibodies (ANCA); therefore, testing for ANCA has no role in the diagnosis of PAN and should not be ordered unless otherwise clinically indicated.

Treatment

The treatment of PAN is guided by the severity of disease. Untreated disease has a uniformly dismal prognosis. Although occasional patients may have "limited" or mild enough disease to warrant treatment only with glucocorticoids, most patients with PAN are treated with a combination of glucocorticoids and a second immunosuppressant Sergent et al. (2009).

In 1996, the French Vasculitis Study Group devised a five-factor score (FFS) (Guillevin et al. 1996) based on patients with PAN, MPA, and Churg-Strauss syndrome (CSS) which included proteinuria >1 g/d, renal insufficiency (stabilized peak creatinine >1.4 mg/dL), cardiomyopathy, severe gastrointestinal manifestations, and CNS involvement. The revised FFS (Guillevin et al. 2011) based on patients with PAN, MPA, CSS, and granulomatosis with polyangiitis (Wegener's) (GPA) found that age >65 years, cardiac symptoms, gastrointestinal involvement, and renal insufficiency (stabilized peak creatinine >1.69 mg/dl) were associated with a high 5-year mortality. The presence of each was assigned 1 point. Ear, nose, and throat (ENT) symptoms, most commonly affecting patients with GPA and CSS, were associated with a lower risk of death, and therefore their absence was assigned 1 point. An FFS = 0 reflects patients without poor prognostic factors and an FFS ≥ 1 suggests the presence of poor prognostic factors that potentially necessitate stronger immunosuppression. Even in patients with an FFS = 0, with a good overall 5-year survival, glucocorticoids have been reported to achieve and maintain remission in only about half of the patients with 40 % patients requiring additional immunosuppressive therapy (Ribi et al. 2010).

Induction therapy for PAN typically involves high-dose glucocorticoids (for the first month followed by a gradual taper) and oral cyclophosphamide (2 mg/kg/day) for a period of 6–12 months. Thereafter, cyclophosphamide is replaced by methotrexate (oral or subcutaneous), azathioprine, or mycophenolate mofetil with continued taper of glucocorticoids for maintenance of immunosuppression. The choice of the maintenance immunosuppressant should be individualized. Informed decision making by the patient in terms of risk and benefit is imperative in the selection of immunosuppression, and close clinical and laboratory monitoring is Stone et al. (2002). In patients with PAN secondary to infection (such as hepatitis B or C or HIV) or malignancy, concomitant appropriate treatment of the primary illness is indicated.

Prognosis

The FFS was also used to derive prognostic information in patients with PAN based on the

severity of illness. Based on the original FFS, scores of 0, 1, and >=2 were associated with 5-year mortality rates of 12 %, 26 %, and 46 %, respectively. According to the revised FFS, 5-year mortality rates for scores of 0, 1, and >=2 were 9 %, 21 %, and 40 %, respectively. In a study comparing outcomes of HBV-associated PAN to idiopathic PAN (Pagnoux et al. 2010), HBV-associated PAN was associated with lower relapse rates but higher all-cause mortality. Age >65 years, hypertension, and gastrointestinal manifestations requiring surgery or surgical consultation were identified as independent predictors of death, whereas patients with cutaneous manifestations or non-HBV-related PAN had a higher risk of relapse.

Conclusion

PAN is a systemic medium-vessel vasculitis that merits prompt recognition and treatment. The choice of immunosuppression is guided by the severity of illness and mandates close monitoring. If the PAN is secondary to another illness, treating the primary illness is of utmost importance. Future research into disease pathogenesis will likely provide us with newer therapies that are safer and more effective.

Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Immune Responses to the Hepatitis C Virus
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Guillevin L, Lhote F, Gayraud M, et al. Prognostic factors in polyarteritis nodosa and Churg-Strauss syndrome. A prospective study in 342 patients. Medicine (Baltimore). 1996;75(1):17–28.

Guillevin L, Pagnoux C, Teixeira L. Polyarteritis nodosa. In: Ball GV, Bridges SL, editors. Vasculitis. Oxford: Oxford University Press; 2008. p. 335–54.

Guillevin L, Pagnoux C, Seror R, et al. The five-factor score revisited: assessment of prognoses of systemic necrotizing vasculitides based on the French vasculitis study group (FVSG) cohort. Medicine (Baltimore). 2011;90(1):19–27.

Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides: the proposal of an international consensus conference. Arthritis Rheum. 1994;37:187–92.

Jennette JC. Cleve Clin J Med. 2002;69 Suppl 2:SII33–8.

Kurland LT, Chuang TY, Hunder G. The epidemiology of systemic arteritis. In: Lawrence RC, Shulman LE, editors. The epidemiology of the rheumatic diseases. New York: Gower; 1984. p. 196–205.

Kussmaul A, Maier R. Ueber eine bisher nicht beschriebene eigenthümliche arterienerkrankung (periarteritis nodosa), die mit morbus brightii und rapid fortschreitender allgemeiner muskellähmung einhergeht. Dtsch Arch Klin Med. 1866;1:484–518.

Pagnoux C, Seror R, Henegar C, et al. Clinical features and outcomes in 348 patients with polyarteritis nodosa: a systematic retrospective study of patients diagnosed between 1963 and 2005 and entered into the French vasculitis study group database. Arthritis Rheum. 2010;62(2):616–26.

Ribi C, Cohen P, Pagnoux C, et al. Treatment of polyarteritis nodosa and microscopic polyangiitis without poor-prognosis factors: a prospective randomized study of one hundred twenty-four patients. Arthritis Rheum. 2010;62(4):1186–97.

Sergent JS. Polyarteritis and related disorders. In: Firestein GS, Budd RC, Harris ED, McInnes IB, Ruddy S, Sergent JS, editors. Textbook of rheumatology. Philadelphia: Saunders Elsevier; 2009. p. 1453–60.

Stone JH. Polyarteritis nodosa. JAMA. 2002;288(13):1632–9.

Polymyalgia Rheumatica

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Definitions

Polymyalgia rheumatica (PMR): Chronic inflammatory condition that affects mainly joints and extra-articular structures of shoulders, neck, and pelvic girdle in individuals after the age of 50.

Definition: Criteria

Polymyalgia rheumatic (PMR) is a chronic inflammatory condition that affects particularly joints and extra-articular structures of shoulders, neck, and pelvic girdle in individuals after the age of 50 (Salvarani et al. 2012). The disease can occur either alone or together with giant cell arteritis (GCA). It has been estimated that approximately 20 % of patients with PMR have GCA, while on the other hand 40–50 % of patients with typical GCA have clinical features of PMR (Salvarani et al. 2012). There have been a number of proposed diagnostic criteria over time for PMR, but none is universally accepted (Salvarani et al. 2012). Recently, the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) have set new classification criteria for the disease (Dasgupta et al. 2012).

Epidemiology

Compared to GCA, PMR is a more common disease with an annual incidence that ranges from 10 to 60 new cases/100,000/year in different countries; the disease is more common in Northern than Southern countries. In the USA, PMR is the most common type of autoimmune inflammatory disease after the age of 50, with an estimated lifetime risk of 2.4 % for women and 1.7 % for men (Crowson et al. 2011). This risk is higher than rheumatoid arthritis (RA) in the same population.

Pathogenesis

Pathological studies have identified varying degrees of inflammation in joints (synovitis) and extra-articular structures (bursitis) in the shoulders, neck, and hips of patients with PMR (Salvarani et al. 2012). The infiltrating cells are mainly CD4+ T cells and macrophages. A similar cellular infiltration is present in temporal arteries of patients with PMR (without GCA), but in the absence of cytokines such as IFN-γ which is usually found in GCA patients. Imaging techniques such as the ^{18}F-FDG-PET scan have identified subclinical inflammation of large arteries in approximately 1/3 of patients with PMR, but in contrast to GCA, vascular complications are rare in PMR patients (Salvarani et al. 2012). The factors that are involved in the initiation of this inflammatory process have not been identified so far.

Clinical Manifestations

The clinical presentation of PMR is characteristic with the abrupt onset of pain and stiffness in both shoulders and often in the neck and hip areas (Salvarani et al. 2012). The symptoms are worse in the morning and night. About one in four patients has pain and swelling of peripheral joints (arthritis) such as the wrists and knees, whereas almost 40 % have systemic manifestations such as low grade fever, weight loss, and fatigue (Salvarani et al. 2012). As mentioned earlier, 20 % of patients can have cranial symptoms of GCA.

Diagnostic Assessments

There are no specific laboratory or imaging tests for the diagnosis of PMR; the diagnosis is clinical and one of exclusion. As in GCA, the majority of patients have elevated erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP) at presentation (Salvarani et al. 2012). Imaging of neck, shoulders, and hips by US, MRI, or ^{18}F-FDG-PET scan reveals synovitis accompanied by prominent bursitis of the affected areas (Salvarani et al. 2012). Unfortunately, these findings are not specific for PMR and can be seen in other inflammatory conditions.

Differential Diagnosis

Late-onset rheumatoid arthritis (RA) is the disease that most commonly mimics PMR (Salvarani et al. 2012). The absence of

rheumatoid factor (RF)/anti-CCP antibodies, joint erosions, and the dramatic response to corticosteroids seen in PMR patients can help in the differential diagnosis. Less frequently, spondyloarthropathies, neoplastic diseases, infections, hypothyroidism, Parkinson's disease, and inflammatory myositis can present with a similar clinical picture.

Treatment

Similarly to GCA, corticosteroids are the first-line treatment for PMR (Salvarani et al. 2012; Dasgupta et al. 2010). A lower initial dose is used (prednisolone 15–20 mg/day) with gradual tapering of the dose. Though the optimal tapering schedule and duration of treatment is uncertain, the treatment duration is commonly 1–2 years; relapses occur in more than half of the cases (Salvarani et al. 2012). Despite the lower cumulative prednisone dose compared to GCA, steroid-induced side effects are frequent occurring in three quarters of patients (Salvarani et al. 2012). Methotrexate and anti-TNF agents have been used as steroid-sparing agents with unclear or limited efficacy (Salvarani et al. 2012).

Given the advanced age of most patients with PMR and their significant corticosteroid exposure, attention to bone health is important. Adequate calcium and vitamin D intake should be routinely encouraged; consideration should also be given to antiresorptive treatment (e.g., a bisphosphonate medication) and monitoring bone mineral density.

Cross-References

- ▶ Giant Cell Arteritis
- ▶ Rheumatoid Arthritis, Clinical Features

References

Crowson CS, Matteson EL, Myasoedova E, Michet CJ, Ernste FC, Warrington KJ, Davis III JM, Hunder GG, Therneau TM, Gabriel SE. The lifetime risk of adult-onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseases. Arthritis Rheum. 2011;63:633–9.

Dasgupta B, Borg FA, Hassan N, Barraclough K, Bourke B, Fulcher J, Hollywood J, Hutchings A, Kyle V, Nott J, Power M, Samanta A. BSR and BHPR guidelines for the management of polymyalgia rheumatica. Rheumatology (Oxford). 2010;49:186–90.

Dasgupta B, Cimmino MA, Maradit-Kremers H, Schmidt WA, Schirmer M, Salvarani C, Bachta A, Dejaco C, Duftner C, Jensen HS, Duhaut P, Poor G, Kaposi NP, Mandl P, Balint PV, Schmidt Z, Iagnocco A, Nannini C, Cantini F, Macchioni P, Pipitone N, Amo MD, Espigol-Frigole G, Cid MC, Martinez-Taboada VM, Nordborg E, Direskeneli H, Aydin SZ, Ahmed K, Hazleman B, Silverman B, Pease C, Wakefield RJ, Luqmani R, Abril A, Michet CJ, Marcus R, Gonter NJ, Maz M, Carter RE, Crowson CS, Matteson EL. Provisional classification criteria for polymyalgia rheumatica: a European league against rheumatism/American college of rheumatology collaborative initiative. Ann Rheum Dis. 2012;71:484–92.

Salvarani C, Pipitone N, Versari A, Hunder GG. Clinical features of polymyalgia rheumatica and giant cell arteritis. Nat Rev Rheumatol. 2012;8:509–21.

Pregnancy in Systemic Lupus Erythematosus

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Synonyms

Fertility; Lupus; Puerperium

Definition

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease which primarily affects women in their reproductive age years. Pregnancy in SLE is associated with increased risks of morbidity and mortality for both the fetus and the mother. These adverse outcomes have well-defined risk factors which are often amenable to modification with preconception planning and careful pregnancy management. The presence of these risk factors also

varies substantially among affected patients. Fortunately, pregnancy can now be managed successfully for most women with SLE. The frequency of pregnancy loss in lupus has dropped over the last 40 years from levels as high as 43 % in 1960–1965 to 17 % in 2000–2003, a level now commensurate with that of the general US population (Clark et al. 2005). In a recent prospective multicenter study of 333 pregnant women with SLE, 80 % had a favorable pregnancy outcome (Buyon et al. 2011). Successful pregnancies in lupus require family planning, preconception counseling, and often management of the patient in a high-risk clinic with the close collaboration of a maternal–fetal medicine specialist and a rheumatologist (Baer et al. 2011; Stojan and Baer 2012).

Pregnancy in Systemic Lupus Erythematosus, Table 1 Risk factors for development of preeclampsia in SLE [Data abstracted from Magee et al. (2008), Stojan and Baer (2012)]

Active lupus nephritis and renal insufficiency at the time of conception
Sustained use of prednisone in doses of 20 mg per day or greater during the pregnancy
Thrombocytopenia
Maternal age $\geq$ 40 years
Previous preeclampsia
Family history of preeclampsia
Antiphospholipid syndrome
Preexisting hypertension or diastolic blood pressure $\geq$ 90 mmHg at first antenatal visit
Preexisting renal disease or proteinuria at first antenatal visit
Preexisting diabetes mellitus
Obesity (body mass index $\geq$ 35 kg/m2)

Pregnancy Outcomes in SLE

The risk of maternal mortality is increased 20-fold, while the risks for maternal morbidity, including cesarean sections, preterm labor, and preeclampsia, are also substantially increased (Clowse et al. 2008). Preeclampsia complicates 13–35 % of lupus pregnancies, compared with 5–8 % of pregnancies in the general US population (Clowse 2007). The risk factors for the development of preeclampsia in SLE are diverse (Table 1).

The rate of pregnancy loss (spontaneous abortion, miscarriage, or stillbirth), preterm birth, and intrauterine growth retardation (IUGR) is higher in women with SLE than in the general obstetric population. A summary of the fetal outcomes of lupus pregnancies in eight-case series is given in Table 2. Adverse fetal outcomes in a lupus pregnancy relate to a variety of maternal disease factors (Table 3).

Flares of Lupus Associated with Pregnancy

A worsening of lupus disease activity (i.e., a flare) occurs during pregnancy or in the immediate postpartum period with reported rates ranging from 13.5 % to 65 % (Molad et al. 2005). Seven prospective comparative studies have examined whether lupus flares are more frequent in pregnant lupus as compared to nonpregnant lupus patients (Table 4) (Stojan and Baer 2012). Four of these studies did not identify an increased rate of flares, while three did. This disparity reflects variability in the severity of lupus among the patients in the study cohorts and in the criteria for defining a lupus flare. More recent prospective studies utilized validated measures of disease activity and found a two- to threefold increase in lupus disease activity during pregnancy (Clowse 2007). The skin, kidney, blood, and joints are the most commonly affected organs in pregnancy-related lupus flares (Clowse 2007). Approximately 15–30 % of patients who flare will have severe disease manifestations, with involvement of the kidneys and other internal organs (Clowse 2007).

Pathophysiology of Lupus Flares During Pregnancy

Estrogens augment immunologic reactivity and mediate in part the increased risk of women for autoimmune disease. A direct role for a sex

Pregnancy in Systemic Lupus Erythematosus, Table 2 Pregnancy and fetal outcomes in SLE

Study	Pregnancies	Live births	Therapeutic abortions	Spontaneous abortions	Fetal deaths	Total pregnancy losses
Mintz et al. (1986)	102	80 (78 %)	0	17 (17 %)	5	22 (22 %)
LêHuong et al. (1997)	62	51 (77 %)	2 (3 %)	10 (16 %)	2 (3 %)	12 (19 %)
Lima et al. (1995)	108	89 (82 %)	2 (2 %)	7 (7 %)	10 (9 %)	19 (18 %)
Georgiou et al. (2000)	59	36 (61 %)	3 (5 %)	9 (15 %)	1 (2 %)	13 (22 %)
Cortés-Hernández et al. (2002)	103	68 (66 %)	8 (8 %)	15 (15 %)	12 (12 %)	35 (34 %)
Liu et al. (2012)	111	83 (75 %)	23 (20 %)	2 (2 %)	8 (9 %)	10 (11 %)
Gladman et al. (2010)	193	114 (59 %)	31 (16 %)	42 (21 %)	3 (2 %)	79 (41 %)
Al Arfaj et al. (2010)	383	269	NA	94 (25 %)	20 (5 %)	114 (30 %)
Ko et al. (2011)	183	152 (83 %)	NA	17 (9 %)	12 (7 %)	29 (16 %)
Clowse et al. (2005)	267	229 (86 %)	NA	19 (7 %)	19 (7 %)	27 (14 %)

Adapted from Stojan and Baer (2012). Complete references available from Stojan and Baer (2012)

Pregnancy in Systemic Lupus Erythematosus, Table 3 Risk factors for pregnancy loss in SLE

Active disease within 6 months prior to conception
Active disease during pregnancy
Systemic lupus erythematosus onset during pregnancy
Secondary antiphospholipid antibody syndrome
Hypocomplementemia
Double-stranded DNA antibodies
Thrombocytopenia
Chronic hypertension
Preexisting renal disease and first-trimester proteinuria (>500 mg/day)

chromosome effect on SLE disease risk has also been demonstrated (Smith-Bouvier et al. 2008). A progressive increase in serum estrogen levels is normal during pregnancy and is thought to promote lupus flares. Interestingly, this increase in estrogen levels was not documented in a study of 17 pregnant lupus patients, possibly as a result of placental compromise (Doria et al. 2002).

Pathologic lesions in the placenta, including vasculopathy, thrombi, and infarction, are common in lupus patients and may underlie the poor gestational outcomes of their pregnancies. Placental weight is generally reduced. These lesions are evident in lupus pregnancies irrespective of the presence of antiphospholipid antibodies. The placental injury may stem from hypercoagulability, hypertension, and immune-mediated vessel damage.

Complement activation may be important to the pathogenesis of preeclampsia, fetal loss, and intrauterine growth restriction. The induction of fetal loss and growth restriction in a murine model of antiphospholipid antibody syndrome required complement activation (Salmon and de Groot 2008). During trophoblast differentiation, phosphatidylserine is externalized on the trophoblast outer leaflet, where it is a target for antiphospholipid antibodies. The binding of these antibodies to the trophoblast, followed by activation of complement, is thought to trigger an inflammatory response with placental injury and adverse effects on the fetus. In pregnant women with or without lupus, the presence of the complement activation product Bb in first-trimester serum samples was a strong predictor of the subsequent development of preeclampsia (Lynch et al. 2008).

Regulatory T cells (T_{REG} cells) are a subset of T lymphocytes that have a key role in the regulation of the immune response and the induction of self-tolerance. They express the surface markers CD4 and CD25 at high levels, as well as a specific T_{REG} marker, the transcription factor forkhead box protein P3 (FOXP3). T_{REG} cells inhibit B, $CD4^+$ and $CD8^+$ T, and natural killer cells and suppress cytokine and antibody production. In SLE, T_{REG} cell numbers are reduced and their function is impaired. T_{REG} cells isolated from patients with SLE have reduced migratory ability and a reduced ability to suppress $CD4^+CD25^-$

Pregnancy in Systemic Lupus Erythematosus, Table 4 Flare rates in SLE

Study	Number of pregnancies	Controls	Flare definition	Pregnancy flares %
Flare rate similar in and out of pregnancy				
1. Lockshin et al. (1984)	33	Matched, nonpregnant	Custom	27 %
2. Mintz et al. (1986)	92	Matched, nonpregnant	Custom	59 %
3. Urowitz et al. (1993)	79	Matched, nonpregnant with and without active SLE	Custom	70 %
4. Tandon et al. (2004)	78	Matched, nonpregnant	Renal activity	45 %
Increased flare rate in pregnancy				
1. Petri et al. (1991)	40	Matched, nonpregnant	PGA	60 %
2. Wong et al. (1991)	29	Nonpregnant	Modified from Lockshin et al. (1984)	58 %
3. Ruiz-Irastorza et al. (1996)	78	Matched, nonpregnant and postpregnancy course	LAI	65 %

Abbreviations: *PGA* physician global assessment, *LAI* Lupus Activity Index
Adapted from Stojan and Baer (2012). Complete references available from Stojan and Baer (2012)

effector T cell proliferation (Tower et al. 2011). The maternal T_{REG} cell population is expanded in murine pregnancy (Aluvihare et al. 2004). Lower levels of T_{REG} cells have been found in human pregnancy decidua obtained from first-trimester spontaneous abortions compared to those from induced abortions (Sasaki et al. 2004), implying that a reduced T_{REG} cell response is involved in abnormal pregnancy. Pregnancy complications in lupus may thus be the result of an intrinsically dysfunctional T_{REG} cell.

Neonatal lupus results from the transplacental passage of maternal SS-A (Ro) and SS-B (La) antibodies that bind to fetal tissue. In the heart, these antibodies trigger inflammation of the atrioventricular nodal and myocardial tissues. Fibrosis ensues, resulting in varying degrees of heart block and occasionally a cardiomyopathy.

Fertility in SLE patients

Women with SLE have normal fertility, unless they are amenorrheic as a result of high disease activity, have advanced renal disease, or have received cyclophosphamide therapy for more severe organ manifestations. Both intravenous pulse and daily oral cyclophosphamide regimens are associated with a high rate of ovarian failure. The prevention of ovarian toxicity during intravenous cyclophosphamide therapy is often attempted with the parallel administration of gonadotropin-releasing hormone agonists (e.g., leuprolide, 3.75 mg, 2 weeks prior to each cyclophosphamide infusion).

Preconception Counseling and Management

The identification of modifiable risk factors for poor pregnancy outcomes in lupus mandates the need for pregnancy planning. Prior to considering pregnancy, a woman with SLE should be advised by her rheumatologist and/or a maternal–fetal medicine specialist as to the risks of both maternal and fetal problems based on her lupus disease activity, comorbidities, and obstetric history. She should receive a specific management plan, including alterations in her medication regimen (if necessary) and monitoring. The outcomes of SLE pregnancies are far better if conception is delayed until more serious lupus disease activity has been absent for at least 6 months and the patient's medication regimen has been adjusted in advance (see below). Women with certain forms of advanced organ damage or disease complications should be advised against considering conception. These include severe pulmonary hypertension, symptomatic restrictive lung

disease, heart failure, chronic kidney disease (serum creatinine 2.8 mg/dl or higher), cerebrovascular accident within the previous 6 months, a history of severe preeclampsia or HELLP despite therapy with aspirin and heparin, and a severe lupus fare within the previous 6 months (Ruiz-Irastorza and Khamashta 2008).

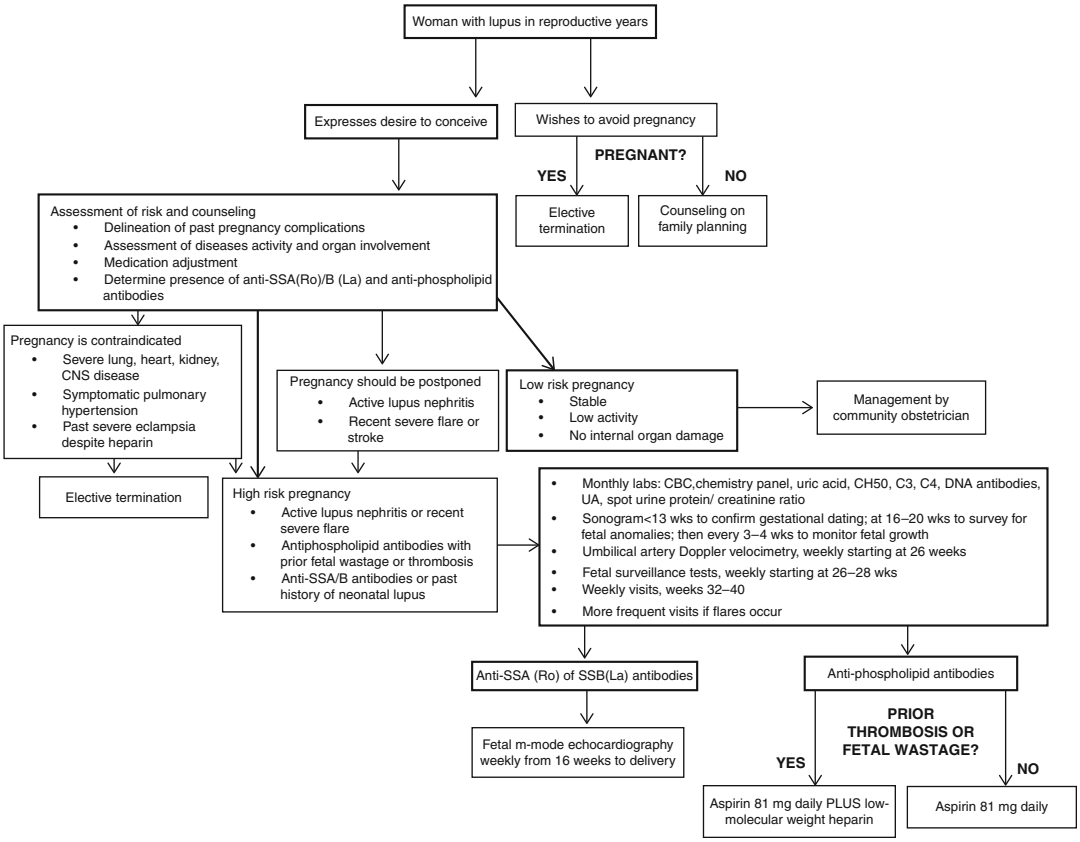
In the absence of any signs or symptoms of active SLE, affected patients require no specific treatment during pregnancy. Immunosuppressive medications with potential teratogenicity, including methotrexate, leflunomide, mycophenolate, and cyclophosphamide, should be stopped at least 3 months prior to conception. Leflunomide needs to be eliminated from the body with an 11-day course of oral cholestyramine, followed by a measurement of the blood level. Hydroxychloroquine is safe and should be continued during pregnancy. In fact, its discontinuation may place the patient at greater risk of increased lupus disease activity during her pregnancy. If there is a need for continued therapy with a nonsteroidal immunosuppressive agent, azathioprine is the safest because the fetal liver cannot metabolize azathioprine into its active form. Low-dose corticosteroid therapy can be maintained if needed for disease control, but the dose should be kept at levels equivalent to prednisone 10 mg daily or less. Higher corticosteroid doses have been reported to increase the risk of hypertension, preeclampsia, preterm birth, premature rupture of membranes, IUGR, and gestational diabetes in lupus patients. However, this has not been evident in the therapy of other conditions.

If antiphospholipid antibodies are present, prophylactic treatment is required to prevent fetal loss. In a first pregnancy, a woman with antiphospholipid antibodies but no history of thrombosis should be treated with low-dose (81 mg) aspirin alone. A woman with antiphospholipid antibodies and a past history of obstetric complications, such as late (>10 weeks) fetal death, IUGR, or preeclampsia, should be treated with low-dose aspirin plus low-molecular-weight heparin as soon as the pregnancy is confirmed. Enoxaparin 30 mg every 12 h or dalteparin 2,500 units every 12 h are used most commonly. Unfractionated heparin (5,000 units every 12 h) is an acceptable alternative. Low-molecular-weight heparin should be switched to unfractionated heparin 4 weeks before the anticipated delivery date. Unfractionated heparin is discontinued at the onset of labor or 8 h before planned cesarean delivery. Heparin is continued for 6 weeks postpartum. Low-dose aspirin alone may be an option for a woman with antiphospholipid antibodies and recurrent early (<10 weeks gestation) miscarriages, but many clinicians would prescribe concomitant heparin.

Diagnostic Testing During Pregnancy

The prenatal care of the pregnant woman with SLE generally requires management in a high-risk clinic and the close collaboration of the obstetrician with a rheumatologist. A detailed algorithm for the obstetric management of the pregnant lupus patient is provided in Fig. 1 and summarized in two recent reviews (Baer et al. 2011; Witter 2007).

At the onset of pregnancy, baseline laboratory tests should include, in addition to the standard registration labs, complete blood count (CBC) and chemistry panel as well as measures of renal function (urinalysis and spot urine protein-to-creatinine ratio) and lupus activity [complement 3 (C3), complement 4 (C4), total hemolytic complement activity (CH50), anti-double-stranded deoxyribonucleic acid (dsDNA)antibodies], estimates of the risk for neonatal lupus [anti-SS-A (Ro), anti-SS-B (La)] and for fetal loss (anticardiolipin antibodies, lupus anticoagulant). A baseline urate level should be obtained since an elevated serum urate level can be a marker of preeclampsia. On a monthly basis throughout the pregnancy, laboratory studies should include a CBC, chemistry panel with serum urate level, urinalysis, spot urine protein/creatinine ratio, C3, C4, CH50, and dsDNA antibodies. If there is protein by urine dipstick and/or an elevated spot urine protein-to-creatinine ratio, then a 24-h urine protein-to-creatinine ratio should be measured.



Pregnancy in Systemic Lupus Erythematosus, Fig. 1 Algorithm for the management of pregnancy in lupus [Figure from (Baer et al. 2011) and reproduced with permission]

A sonogram with measurement of crown-rump distance should be performed early in pregnancy to confirm gestational dating and again at 16–20 weeks of gestation, to survey for fetal anomalies, and to monitor fetal growth. Subsequent sonograms should be done at 4-week intervals or every 3 weeks in the setting of preeclampsia or suspected IUGR to monitor fetal growth and measure amniotic fluid volume. Uterine artery Doppler studies serve to define the adequacy of uteroplacental blood flow and may have utility as a predictor of preeclampsia. The first such study may be done around the 20th week of gestation and if abnormal, repeated 4 weeks later.

Fetal surveillance tests should be started at 26–28 weeks and continued weekly until birth. These include the nonstress test (NST) which can begin at 28 weeks, the biophysical profile which can begin at 26 weeks without the NST, and fetal umbilical artery Doppler velocimetry which can be begin at 26 weeks. Abnormal umbilical and uterine arterial blood flow assessed with Doppler velocimetry during the second trimester may be predictive of preeclampsia and IUGR. Detection of IUGR with these fetal surveillance tests should prompt closer monitoring and consideration of early delivery.

In utero heart block develops as a manifestation of neonatal lupus after 16 weeks of gestation. Most cases occur between 16 and 24 weeks, but some occur as late as 38–40 weeks. Since the early recognition of heart block in the fetus may allow for therapeutic intervention, close monitoring of at-risk fetuses is mandatory. The fetal heart rhythm should be monitored by m-mode echocardiography weekly from 16 weeks onward.

Recognition of a Lupus Flare During Pregnancy

Medical complications of a lupus pregnancy include lupus flares with or without glomerulonephritis, hypertension, preeclampsia, and the hemolysis, elevated liver enzymes, and low platelet counts (HELLP) syndrome (Stojan and Baer 2012). Recognition of a lupus flare during pregnancy may be difficult since the signs and symptoms may mimic those of normal pregnancy. Examples of this apply to most organ systems (Table 5). Severe fatigue, facial and palmar erythema, melasma, postpartum hair loss, dyspnea, arthralgias, and headaches frequently accompany normal pregnancy. Bland joint effusions occur in late pregnancy. The serum creatinine level is decreased as a result of increased renal blood flow and glomerular filtration rate. A serum creatinine of >0.8 mg/dl and blood urea nitrogen (BUN) >13 mg/dl are considered indicative of renal impairment in pregnancy. A creatinine level that remains stable throughout pregnancy and does not decrease can also be a sign of renal insufficiency. The increased renal blood flow of pregnancy results in increased tubular flow and urine protein spillage. Levels of proteinuria up to 300 mg/day are considered normal in pregnancy. In women with prior renal damage from lupus nephritis, the degree of urine protein loss may increase. However, a doubling in the amount of the baseline urine protein should be a cause for concern. Hepatic protein synthesis also increases in pregnancy, and this can result in higher levels of the serum complement components as well as fibrinogen. The latter may lead to a substantial increase in the erythrocyte sedimentation rate in a normal pregnancy.

Assessing Lupus Activity During Pregnancy

The established lupus activity scales, for example, the Lupus Activity Index (LAI), SLE Disease Activity Index (SLEDAI), and Systemic Lupus Activity Measure (SLAM), were validated with populations that excluded pregnant women and included men. Their shortcomings

Pregnancy in Systemic Lupus Erythematosus, Table 5 Assessment of lupus flares in pregnancy

Feature	Findings indicative of a lupus flare	Findings of normal pregnancy that can mimic a flare
Clinical	• Active rash of lupus • Inflammatory arthritis • Lymphadenopathy • Fever >38 °C (not related to infection or drug) • Pleuritis • Pericarditis	• Fatigue • Arthralgias • Bland effusions of knees • Myalgias • Malar and palmar erythema • Postpartum hair loss • Carpal tunnel syndrome • Edema of hands, legs, and face • Mild resting dyspnea
ESR	Increased	18–46 mm/h < 20 weeks gestation
		30–70 mm/h ≥ 20 weeks gestation
Anemia	Hemoglobin <10.5 g/dl	Hemoglobin >11 g/dl during first 20 weeks gestation
		Hemoglobin >10.5 g/dl hr after 20 weeks gestation
Thrombocytopenia	Platelet count <95,000	Mild in approximately 8 %
Urinalysis	Hematuria or cellular casts	Rare hematuria from vaginal contamination
Proteinuria	≥300 mg/d	<300 mg/dl
dsDNA antibodies	Rising titers	Negative or stable titers
Complement	≥25 % drop	Usually increased

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in distinguishing between physiologic changes seen in pregnancy and true lupus manifestations led to the development of the LAI-P and SLEPDAI, lupus activity scales specific for pregnant women (Stojan and Baer 2012).

Differentiation of Preeclampsia from a Lupus Nephritis Flare

Preeclampsia is defined as a blood pressure >140/90 mmHg and proteinuria >300 mg in a 24-h urine specimen detected for the first time after 20 weeks of gestation. It is often difficult to differentiate preeclampsia from lupus nephritis, since both entities may present with increasing proteinuria, hypertension, lower extremity edema, deterioration in renal function, and thrombocytopenia. The two conditions may coexist.

Features that distinguish preeclampsia from active lupus nephritis are summarized in Table 6. These include a serum uric acid >5.5 mg/dl, a urine calcium level of <195 mg/day, and rising liver enzyme levels. Features of active lupus nephritis include a rise in dsDNA antibody titer, low or dropping complement levels, increased lupus activity in other organs, and active urinary sediment. A renal biopsy may be needed to define the presence of active lupus glomerulonephritis; however, consideration should be given to the increased risk of bleeding following such biopsies in pregnancy. On occasion, this diagnostic dilemma can only be resolved with delivery of the fetus or a trial of empiric therapy.

Pregnancy in Systemic Lupus Erythematosus, Table 6 Differentiation of active lupus nephritis from preeclampsia

	Active lupus nephritis	Preeclampsia
Hypertension	Onset before 20 weeks	Onset after 20 weeks
Proteinuria	≥300 mg/d	≥300 mg/dl
Urinary sediment	Active	Inactive
Uric acid	≤5.5 mg/dl	>5.5 mg/dl
DNA antibody levels	Rising	Stable or negative
24-h urine calcium	≥195 mg/day	<195 mg/day
Complement levels	≥25 % drop	Normal

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Therapy of Lupus Flares During Pregnancy

The management of lupus flares during pregnancy depends on the severity and type of organ involvement. Nonsteroidal anti-inflammatory drug (NSAID) use after 20 weeks of pregnancy is avoided since it has been associated with premature closure of the patent ductus arteriosus, reversible oligohydramnios, gastrointestinal bleeding or perforation, increased risk of necrotizing enterocolitis, pulmonary hypertension, intracranial bleeding, and prolongation of labor. Hydroxychloroquine decreases the incidence of flares and should be maintained throughout pregnancy, particularly if it was being used prior to conception. It is generally effective for arthritis and skin manifestations of lupus. Lupus flares that are not adequately controlled with acetaminophen and hydroxychloroquine require corticosteroids. Anemia (hemoglobin <8 g/dl), sustained fever (>38.5 °C), and low serum albumin levels (<3 g/dl) threaten the developing fetus and demand more aggressive therapy (Lockshin and Sammaritano 2003). Prednisone and prednisolone are the preferred corticosteroids in pregnancy because they are readily inactivated by the placenta. The fluorinated glucocorticoids, dexamethasone and betamethasone, cross the placenta easily without being inactivated and should only be used if there is intent to treat the fetus, such as treating in utero heart block or inducing fetal lung maturation before preterm delivery. Low doses (<20 mg/day) of prednisone are used to treat mild lupus activity, while pulse doses of intravenous methylprednisolone are used to treat more severe lupus activity. There may be a twofold increased risk for cleft lip or palate with systemic corticosteroid use in the first trimester, although the absolute risk remains low (Pradat et al. 2003). Important adverse effects in pregnancy include hypertension, osteopenia, osteonecrosis,

susceptibility to infection, and an increased risk of gestational diabetes.

In order to minimize steroid exposure of patients with moderate to severe lupus activity, long-term management with a second-line, "steroid-sparing" agent may be needed. The preferred drug in this category is azathioprine at a daily dose not exceeding 2 mg/kg, although its use during pregnancy has been associated with IUGR and an increased rate of pregnancy loss. Cyclosporine is safe for the fetus and can be used to treat renal disease during pregnancy but poses risks of maternal nephrotoxicity. Many successful pregnancies have been reported in women with solid organ transplants who continue treatment with cyclosporine and tacrolimus during pregnancy, without any increased risk of congenital abnormalities when compared to the general population. Intravenous immunoglobulin has no known adverse effects on the fetus and is used for the treatment of thrombocytopenia during pregnancy. Mycophenolate is teratogenic and should be avoided during pregnancy.

Severe manifestations of lupus that do not respond to other therapies may require cyclophosphamide, with the understanding that the fetus may not survive without treatment of the mother. First-trimester use is associated with fetal malformations, whereas use in the second and third trimesters has been associated with growth retardation and suppression of fetal hematopoiesis.

Animal data on the safety of belimumab are encouraging since teratogenic effects were not identified. Due to insufficient data regarding fetal exposure to belimumab, the current Food and Drug Administration recommendations are to discontinue belimumab 4 months prior to anticipated conception. Rituximab is detectable in serum 3–6 months after completion of the treatment infusions and has been detected in the serum of infants exposed in utero. Studies of children who were exposed to rituximab early in pregnancy have not demonstrated any adverse outcomes to date; however, second- and third-trimester exposure causes B cell depletion in the fetus with unknown long-term effects. Although congenital malformations and neonatal infections were rarely seen among exposed neonates, women should continue to be counseled to avoid pregnancy for 6–12 months after rituximab exposure.

The optimal treatment for in utero heart block in a mother with anti-SS-A (Ro) or SS-B (La) antibodies has not been established. The use of hydroxychloroquine during pregnancy is associated with a reduced risk of fetal heart block, while intravenous immunoglobulin therapy does not reduce the incidence of fetal heart block in high-risk pregnant women with a previous pregnancy complicated by this disease (Pisoni et al. 2010). The initiation of maternal oral dexamethasone (4–8 mg/day for 2 weeks, followed by 4 mg/day) therapy is recommended at the first indication of heart block due to a significant reduction in morbidity and improved outcomes of routinely treated fetuses.

Conclusion

Successful pregnancies can be realized for the majority of women with systemic lupus erythematosus. However, the management of such pregnancies requires careful preconception planning and advanced adjustment of medications. The best outcomes for a lupus pregnancy occur when the disease has been in remission for at least the 6 months prior to conception.

Cross-References

- ▶ Anti-phospholipid Antibody Mechanisms of Thrombosis
- ▶ Antiphospholipid Syndrome, Clinical Manifestations
- ▶ Antiphospholipid Syndrome Treatment
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Systemic Lupus Erythematosus, Gender and Hormone Influences
- ▶ Systemic Lupus Erythematosus, Genetics
- ▶ Tregs in the Liver

References

Al Arfaj AS, Khalil N. Pregnancy outcome in 396 pregnancies in patients with SLE in Saudi Arabia. Lupus. 2010;19(14):1665–73.

Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004;5(3):266–71.

Baer AN, Witter FR, Petri M. Lupus and pregnancy. Obstet Gynecol Surv. 2011;66(10):639–53.

Buyon JP, Garabet L, Kim M, Reeves ER, Guerra MM, Lockshin MD, et al. Favorable prognosis in a large, prospective multicenter study of lupus pregnancies. Arthritis Rheum. 2011;63(10):S669.

Clark CA, Spitzer KA, Laskin CA. Decrease in pregnancy loss rates in patients with systemic lupus erythematosus over a 40-year period. J Rheumatol. 2005;32(9):1709–12.

Clowse ME, Magder LS, Witter F, Petri M. The impact of increased lupus activity on obstetric outcomes. Arthritis Rheum. 2005;52(2):514–21.

Clowse ME. Lupus activity in pregnancy. Rheum Dis Clin North Am. 2007;33(2):237v–52.

Clowse ME, Jamison M, Myers E, James AH. A national study of the complications of lupus in pregnancy. Am J Obstet Gynecol. 2008;199(2):127.e1–127.e6.

Cortés-Hernández J, Ordi-Ros J, Paredes F, Casellas M, Castillo F, Vilardell-Tarres M. Clinical predictors of fetal and maternal outcome in systemic lupus erythematosus: a prospective study of 103 pregnancies. Rheumatology (Oxford). 2002;41(6):643–50.

Doria A, Cutolo M, Ghirardello A, Zampieri S, Vescovi F, Sulli A, et al. Steroid hormones and disease activity during pregnancy in systemic lupus erythematosus. Arthritis Rheum. 2002;47(2):202–9.

Georgiou PE, Politi EN, Katsimbri P, Sakka V, Drosos AA. Outcome of lupus pregnancy: a controlled study. Rheumatology (Oxford). 2000;39(9):1014–9.

Gladman DD, Tandon A, Ibañez D, Urowitz MB. The effect of lupus nephritis on pregnancy outcome and fetal and maternal complications. J Rheumatol. 2010;37(4):754–8.

Ko HS, Ahn HY, Jang DG, et al. Pregnancy outcomes and appropriate timing of pregnancy in 183 pregnancies in Korean patients with SLE. Int J Med Sci. 2011;8(7):577–83.

Lê Huong D, Wechsler B, Vauthier-Brouzes D, et al. Outcome of planned pregnancies in systemic lupus erythematosus: a prospective study on 62 pregnancies. Br J Rheumatol. 1997;36(7):772–7.

Lima F, Buchanan NM, Khamashta MA, Kerslake S, Hughes GR. Obstetric outcome in systemic lupus erythematosus. Semin Arthritis Rheum. 1995;25(3):184–92.

Liu J, Zhao Y, Song Y, et al. Pregnancy in women with systemic lupus erythematosus: a retrospective study of 111 pregnancies in Chinese women. J Matern Fetal Neonatal Med. 2012;25(3):261–6.

Lockshin MD, Sammaritano LR. Lupus pregnancy. Autoimmunity. 2003;36(1):33–40.

Lockshin MD, Reinitz E, Druzin ML, Murrman M, Estes D. Lupus pregnancy. Case–control prospective study demonstrating absence of lupus exacerbation during or after pregnancy. Am J Med. 1984;77(5):893–8.

Lynch AM, Murphy JR, Byers T, Gibbs RS, Neville MC, Giclas PC, et al. Alternative complement pathway activation fragment bb in early pregnancy as a predictor of preeclampsia. Am J Obstet Gynecol. 2008;198(4):385.e1–385.e9.

Magee LA, Helewa M, Moutquin JM, von Dadelszen P. Hypertension Guideline Committee, Strategic Training Initiative in Research in the Reproductive Health Sciences (STIRRHS) Scholars. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy. J Obstet Gynaecol Can. 2008;30(3 Suppl):S1–48.

Mintz G, Niz J, Gutierrez G, Garcia-Alonso A, Karchmer S. Prospective study of pregnancy in systemic lupus erythematosus. Results of a multidisciplinary approach. J Rheumatol. 1986;13(4):732–9.

Molad Y, Borkowski T, Monselise A, Ben-Haroush A, Sulkes J, Hod M, et al. Maternal and fetal outcome of lupus pregnancy: a prospective study of 29 pregnancies. Lupus. 2005;14(2):145–51.

Petri M, Howard D, Repke J. Frequency of lupus flare in pregnancy. The Hopkins Lupus Pregnancy Center experience. Arthritis Rheum. 1991;34(12):1538–45.

Pisoni CN, Brucato A, Ruffatti A, Espinosa G, Cervera R, Belmonte-Serrano M, et al. Failure of intravenous immunoglobulin to prevent congenital heart block: findings of a multicenter, prospective, observational study. Arthritis Rheum. 2010;62(4):1147–52.

Pradat P, Robert-Gnansia E, Di Tanna GL, Rosano A, Lisi A, Mastroiacovo P, et al. First trimester exposure to corticosteroids and oral clefts. Birth Defects Res A Clin Mol Teratol. 2003;67(12):968–70.

Ruiz-Irastorza G, Khamashta MA. Lupus and pregnancy: ten questions and some answers. Lupus. 2008;17(5):416–20.

Ruiz-Irastorza G, Lima F, Alves J, et al. Increased rate of lupus flare during pregnancy and the puerperium: a prospective study of 78 pregnancies. Br J Rheumatol. 1996;35(2):133–8.

Salmon JE, de Groot PG. Pathogenic role of antiphospholipid antibodies. Lupus. 2008;17(5):405–11.

Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4 + CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod. 2004;10(5):347–53.

Smith-Bouvier DL, Divekar AA, Sasidhar M, Du S, Tiwari-Woodruff SK, King JK, et al. A role for sex chromosome complement in the female bias in autoimmune disease. J Exp Med. 2008;205(5):1099–108.

Stojan G, Baer AN. Flares of systemic lupus erythematosus during pregnancy and the puerperium:

prevention, diagnosis and management. Expert Rev Clin Immunol. 2012;8(5):439–53.
Tandon A, Ibañez D, Gladman DD, Urowitz MB. The effect of pregnancy on lupus nephritis. Arthritis Rheum. 2004;50(12):3941–6.
Tower C, Crocker I, Chirico D, Baker P, Bruce I. SLE and pregnancy: the potential role for regulatory T cells. Nat Rev Rheumatol. 2011;7(2):124–8.
Urowitz MB, Gladman DD, Farewell VT, Stewart J, McDonald J. Lupus and pregnancy studies. Arthritis Rheum. 1993;36(10):1392–7.
Witter FR. Management of the high-risk lupus pregnant patient. Rheum Dis Clin North Am. 2007 May;33(2):253,65, v-vi.
Wong KL, Chan FY, Lee CP. Outcome of pregnancy in patients with systemic lupus erythematosus. A prospective study. Arch Intern Med. 1991;151(2):269–73.

Primary Biliary Cirrhosis, Overview

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Synonyms

Additional medical therapy; Liver transplantation

Definition

Primary biliary cirrhosis (PBC) is a chronic inflammatory autoimmune disease that targets mainly the cholangiocytes of the interlobular bile ducts in the liver. The condition affects primarily middle-aged women. Without treatment, PBC generally progresses to cirrhosis and eventually liver failure over a period of 10–20 years.

The term "primary biliary cirrhosis" was coined to distinguish the disease from obstructive biliary cirrhosis by Dauphinée and Sinclair in 1949 (Dauphinee and Sinclair 1949) and used by Arhens and colleagues in 1950 in their description of the disease affecting middle-aged to older women with progressive jaundice, pruritus, and hepatosplenomegaly (Ahrens et al. 1950). In 1965, Rubin and colleagues coined the fundamental histological lesion as non-suppurative destructive cholangitis (NSDC) (Rubin et al. 1965). The disease-specific hallmark – the antimitochondrial antibody (AMA) – was identified the same year by Walker and colleagues, a discovery that allowed a confident diagnosis earlier in the course of the disease (Walker et al. 1965). Among the various mitochondrial antigens, the so-called M2 was shown latter to be specifically associated with PBC (Berg et al. 1982). Five years later, the cDNA for the major mitochondrial antigen of PBC was cloned from gene expression library and identified as the pyruvate dehydrogenase complex (Gershwin et al. 1987).

Epidemiology

The incidence and prevalence of PBC have increased over time. Most recent data in Europe, North America, and Japan show an incidence of 2–4 per 100,000 population per year and point prevalence at 20–40 cases per 100,000 (Poupon 2010). Geographic clustering is striking, suggesting genetic as well as environmental influences in its pathogenesis. Whether the higher incidence and prevalence rates reflect a genuine increase or whether they are due to a higher detection rate remains unknown.

Causes and Pathogenesis

PBC is thought to be a complex disease resulting from the combination of multiple genetic factors and superimposed environmental triggers (Poupon 2010). These factors may affect one or more components of the immune system and of the tissue targeted by the disease. Genetic predisposition is suggested by the familial clustering of the disease and the high degree concordance between monozygotic twins. The prevalence of PBC is 100 times higher in first-degree relatives

than in the general population. Allelic variations in MHC class II (DR,DQ), components of the innate (C4*Q0,C4B*2,NRAMP1/SLC11A1, MBL,VDR) and adaptive (CTLA4, Il beta, TNF alpha, IL12A, IL12RB2) immune systems, have been associated with PBC susceptibility. The genetic basis of variability in disease progression is poorly known. The French prospective study of genetic factors of PBC severity has suggested a role for TNF alpha and SLCA2/AE2 variants. Regarding environmental factors, mucosal infections, particularly urinary tract infections, and cigarette smoking have been consistently associated with PBC. Smoking not only predisposes to disease but may also accelerate its progression. The role of xenobiotics through covalent binding of 2-octynoic acid, a component of many chemicals, to proteins is suggested by experimental studies. The ratio of women to men affected by the disease is 10 to 1.

Clinical Presentation

There are three major forms of PBC. The typical or classical form is represented by the slowly progressive decline of small bile ducts and parallel increase in liver fibrosis, leading to biliary cirrhosis over a period of 10–20 years. A second form that affects 10–20 % of patients is characterized by the fluctuating or persistent presence of autoimmune hepatitis (AIH) features. These patients have a more severe course, with early development of hepatic fibrosis and liver failure. A third form that affects 5–10 % of patients is represented by the so-called premature ductopenic variant. Its hallmark is a very rapid onset of ductopenia, severe icteric cholestasis, and hyperlipidemia, progressing toward cirrhosis and liver failure in less than 5 years.

PBC is now diagnosed earlier in its clinical course than it was in the past. Half of the patients are asymptomatic at diagnosis. Fatigue and pruritus are the two symptoms of the early phase of the disease. Fatigue, a frequent complaint, is unrelated to the severity. Whether it is a specific symptom or not remains unknown. It is associated with cognitive and emotional dysfunction, depression, and sensory and autonomic abnormalities. It may be associated with excessive day-time somnolence. A mild pruritus affects about half the patients at diagnosis. This pruritus might be severe and affect the quality of life in the premature ductopenic variant.

In some patients, the diagnosis is made in the work-up of another autoimmune disease.

In 5 to 10 of the patients, the diagnosis is made at the stage of compensated or decompensated cirrhosis. The variant associated with AIH features might be misdiagnosed for acute cytolytic hepatitis.

Biochemistry of the typical form of the disease is predominantly characterized by elevation of serum alkaline and gamma-glutamyltranspeptidase activities while serum transaminases are mildly or only moderately increased. In the premature ductopenic variant of the disease, cholestasis is prominent and associated with considerable hypercholesterolemia affecting both HDL, LDL, and LPX fractions. In patients with both AIH and PBC features, serum transaminase activities may be markedly elevated and usually associated with marked elevation of IgG and presence of smooth muscle antibodies. Increased serum IgM level is detected in 80 % of the patients. Thrombocytopenia, polyclonal hyperglobulinemia, and hyperbilirubinemia are indices of cirrhosis. Low prothrombin index and hypoalbuminemia are usually observed in the late phase of the disease. However, mild hypoalbuminemia, occurring possibly as a result of the inflammatory process, may be present in the absence of cirrhosis in some patients with early stage disease.

Diagnostic Investigation

Good ultrasound investigation of the liver and biliary tree is mandatory in all patients with liver abnormalities. If ultrasound suggests a normal biliary system and if the patient is AMA type M2 positive, no further radiologic delineation of the bile ducts is necessary. Cholangiography might be necessary in the following settings: atypical presentation even in

case of AMA positivity, AMA negativity, and suspicion of comorbidity affecting the biliary tract.

A major PBC hallmark is the presence of serum AMA type M2. The AMA that reacts with the E2 component of pyruvate dehydrogenase serves as a diagnosis for PBC. A variety of antinuclear antibodies are associated with PBC. Antibodies recognizing proteins of the pore complex (gp 210, nucleoporin 62) and nuclear body protein (sp100) are almost totally specific.

Liver histologic examination is only mandatory for the diagnosis if the AMA test is negative or if the patient displays the atypical biochemical sign of PBC or is suspected of superimposed comorbidity. Nevertheless, it should be emphasized that histology is valuable for prognostic evaluation and treatment strategy.

The three following criteria allow PBC diagnosis: (a) abnormal biochemical tests with preferential elevation of serum alkaline phosphatase and gamma-glutamyltranspeptidase activities; (b) presence of antimitochondrial antibodies with M2 specificity by ELISA or immunoblotting; (c) histological evidence of LDC. These three criteria are sufficient for diagnosis considering the high specificity of anti-M2 antibody and LDC.

Patients with AMA and normal biochemical tests may be at risk of developing true PBC and their liver function tests should be reassessed annually.

AMA negative patients with biochemical evidence of cholestasis may have PBC if LCD or portal inflammation and ductopenia are observed in liver biopsies. The diagnosis is further supported in this setting if antinuclear antibodies against gp210, Nucleoporin 62, sp100 giving nuclear-rim or nuclear-dot pattern are present. LDC is highly suggestive of PBC but is not pathognomonic since it may be present in patients with AIH, primary sclerosing cholangitis, lymphoma, and viral hepatitis C and E.

AIH variant forms might display several concurrent PBC features and be difficult to diagnose. Antibodies specific for the M2 autoantigen could be found in a minority of AIH patients. Up to 24 % of AIH patients might display histological bile duct injury without biochemical cholestasis but they respond to AIH conventional therapy. Finally, up to 20 % of PBC patients might have features of AIH at presentation or during the follow-up (Poupon 2003). In this context, the so-called PBC-AIH overlap syndrome is defined by the simultaneous or consecutive concurrent main characteristics of the two conditions. The following score has been proposed to establish the diagnosis, e.g., presence of two of the three following features: (1) ALT activity $\geq$ 5 times upper limits of normal; (2) IgG $\geq$ 2 times upper limits of normal and/or positive anti-smooth muscle antibody; and (3) liver biopsy with moderate or severe periportal or periseptal inflammation. Patients with PBC-AIH overlap syndrome have a more severe disease compared to patients with PBC alone.

Treatment

Specific Therapy

UDCA Therapy

All PBC patients with abnormal liver function tests should be considered for therapy (Poupon 2010, 2003; European Association for the Study of the Liver 2009; Lindor et al. 2009). Daily treatment with 13–15 mg/kg of UDCA is currently considered the mainstay of PBC therapy. Randomized, double-blinded, placebo-control trials have consistently shown that UDCA improves liver function test parameters including serum bilirubin, the major prognostic marker in PBC. UDCA delays the progression of fibrosis and liver damage. A combined analysis of three randomized-controlled trials including 548 PBC sufferers showed that UDCA treatment for up to 4 years led to improved survival of patients with moderate to severe disease. These patients did not require liver transplantation. Long-term studies have shown that UDCA therapy provides a better survival than that predicted by the Mayo model. The survival rate of UDCA-treated patients at an early stage of the disease is similar to that of a control healthy population. In both Europe and

North America, the number of liver transplants for PBC is falling in parallel with increasing use of UDCA therapy. A meta-analysis that included short-term trials with low doses of UDCA (less than 12 mg/kg/day) has questioned UDCA efficacy. However, on the basis of all available data, it is currently recommended to treat PBC with UDCA using doses of at least 13 mg/kg/day and to start early. The aim of UDCA therapy is normalize serum bilirubin, alkaline phosphatase, and ALT or AST levels during the first year of therapy. Patients with normalized liver function test parameters have a null risk to progress to cirrhosis. This could not, however, be achieved in all patients. To be effective, UDCA should ideally be used to treat patients with serum bilirubin levels less than 1 mg/dl and AST levels less than twice the upper normal limit of normal, and alkaline phosphatase less than three times the upper normal limit of normal at the end of the first year of therapy. Indeed, the patients with these criteria have a 10-year life expectancy without liver transplantation similar to that of the control population. Other studies have reported that patients showing a > 50 % decline in serum alkaline phosphatase levels have an excellent long-term prognosis. About 40 % of patients have a suboptimal response to UDCA. These patients need an adjuvant therapy.

Adjuvant Therapies

Patients with features of AIH, severe interface hepatitis, abnormal serum bilirubin levels, or suboptimal response to UDCA (as defined above) deserve trials with adjuvant therapies. However, there is no consensus on how to treat these patients.

As stated by the EASL Clinical Practice Guidelines, combined therapy with UDCA and steroids is the recommended therapeutic option for patients with PBC-AIH overlap syndrome.

Glucocorticoids (prednisone or budesonide) and methotrexate could be also considered for patients with incomplete response and ongoing liver inflammation and destructive cholangitis. The rationale for the use of glucocorticoids is based on the following arguments. Glucocorticoids and specifically budesonide have been shown to be beneficial for UDCA-treated patients. Combination of both treatments improves liver function tests and decreases inflammation and fibrosis. The drug is, however, contraindicated for patients with cirrhosis. Patients with a suboptimal response to UDCA and marked periportal inflammation benefit from a treatment combining UDCA and glucocorticoids (in particular budesonide). In this setting, steroid-sparing agents, such as azathioprine or mycophenolate mofetil, could be used.

Methotrexate improved biochemical test results and liver histological findings when it was added to UDCA in patients who had marked portal and periportal inflammation and incomplete response to UDCA. However, other studies found no efficacy of methotrexate when used alone or in combination with UDCA. Several drugs, including cyclosporine, colchicine, chlorambucil, penicillamine, azathioprine, mycophenolate mophetil, malotilate, and thalidomide, have been evaluated for the treatment of PBC. Many of them are either ineffective or toxic. None of them have been shown to be effective in UDCA-treated patients with a risk of developing cirrhosis or liver failure as defined above.

New Medical Therapeutic Options

FXR is a major bile acid receptor that regulates the expression of genes involved in bile acid and lipid metabolism. It may also modulate innate immunity in the biliary tree and intestine. The FXR agonist, obeticholic acid, improved liver function tests and reduced IgM levels in PBC. The efficacy of this drug is currently assessed in a phase 3 clinical trial.

PPAR alpha agonists (bezafibrate, fenofibrate) are now recognized for their anti-inflammatory and immunomodulatory properties in several experimental models of autoimmunity. Short-term administration of fibrates leads to marked improvement of liver biochemistries and immunoglobulin levels in PBC. Whether it could improve liver inflammation and fibrosis remains unknown.

Liver Transplantation

Liver transplantation has greatly improved survival in patients with PBC (MacQuillan and Neuberger 2003). It is the only effective treatment for those with decompensated cirrhosis or liver failure. The patients featuring the premature ductopenic variant of PBC do not respond to any medical therapy and despite the absence of any decompensation draw a major benefit from liver transplantation. PBC recurs in about 20 % of patients at 5 years. Recurrence is more frequent in patients without a glucocorticoid and ciclosporin regimen. The beneficial long-term effect of UDCA in this setting remains unknown.

Treatment of Symptoms and Complications

Pruritus

Cholestyramine is widely used as first-line treatment although the evidence to support this use is limited. When both UDCA and cholestyramine are used, each drug should be administered at a minimum of 4 h apart to prevent binding and loss of efficacy. Although there is strong evidence that Rifampicin at a daily dosage of up 600 mg/day is effective to treat patients receiving UDCA, this drug induces occasionally hepatitis. Other therapies include glucocorticoids, sertraline, and opiate antagonists. Plasmapheresis or biliary drainage may be successful when other treatments fail. In very few patients, resistant pruritus may be an indication for liver transplantation.

Fatigue

The fatigue could be multifactorial and causes other than PBC should be considered. Modafinil, a drug approved for the treatment of narcolepsy, has been reported in open studies to provide significant benefit for PBC patients with fatigue. The drug used at a dose up to 400 mg/day seems to be well tolerated and seems effective in those with excessive fatigue and day-time somnolence.

Hypercholesterolemia

UDCA induces an average 15–20 % fall in total and LDL cholesterol at 1 year of therapy. Statins and fibrates are safe and effective in PBC.

Portal Hypertension

A minority of patients with PBC develop presinusoidal portal hypertension before developing cirrhosis. The management of portal hypertension in patients with PBC should be as for all cirrhotic patients. Severe portal hypertension even without any other sign of decompensation is a good indication of liver transplantation.

Osteopenia and Osteoporosis

Current treatments for osteopenia and osteoporosis that affect up to 30 % of PBC patients included physical activity, calcium, and vitamin D supplementation in order to achieve at least 30 ng/ml of serum 25OH D3, bisphosphonates or estrogens supplementation.

Conclusion

- Primary biliary cirrhosis is a chronic inflammatory autoimmune disease that targets mainly the cholangiocytes of the interlobular bile ducts.
- PBC is a rare disease that affects primarily middle-aged women.
- Risks factors include history of familial autoimmune disease, history of active or passive smoking, and recurrent urinary tract infections.
- PBC is associated with features of autoimmune hepatitis in 10 % of the patients.
- Diagnosis of PBC can be established when two of the following three criteria are met: unexplained chronic elevation of alkaline phosphatase, presence of AMA type 2, or histologic evidence of lymphocytic destructive cholangitis.
- Without treatment, PBC may progress to cirrhosis and eventually liver failure over of a 10–20-year period.
- Ursodeoxycholic acid is currently the only FDA-approved medical treatment for PBC. When administered at adequate doses of 13–15 mg/kg/day, a majority of the patients with PBC may have a normal life expectancy without additional therapeutic measures.

- One out of three patients does not adequately respond to UDCA therapy and may need adjuvant therapies.

Cross-References

- ▶ Autoimmune Hepatitis
- ▶ CD5
- ▶ Environment and Autoimmunity
- ▶ Immunosuppression in Clinical Liver Transplantation
- ▶ PBC Genetics
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Ahrens Jr EH, Payne MA, Kunkel HG, Eisenmenger WJ, Blondheim SH. Primary biliary cirrhosis. Medicine (Baltimore). 1950;29:299–364.

Berg PA, Klein R, Lindenborn-Fotinos J, Kloppel W. ATPase-associated antigen (M2): marker antigen for serological diagnosis of primary biliary cirrhosis. Lancet. 1982;2:1423–6.

Dauphinee JA, Sinclair JC. Primary biliary cirrhosis. Can J Gastroenterol. 1949;61:1–6.

European Association for the Study of the Liver. EASL clinical practice guidelines: management of cholestatic liver diseases. J Hepatol. 2009;51:237–67.

Gershwin ME, Mackay IR, Sturgess A, Coppel RL. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol. 1987;138:3525–31.

Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. Hepatology. 2009;50:291–308.

MacQuillan GC, Neuberger J. Liver transplantation for primary biliary cirrhosis. Clin Liver Dis. 2003;7:941–56.

Poupon R. Autoimmune overlapping syndromes. Clin Liver Dis. 2003;7:865–78.

Poupon R. Primary biliary cirrhosis : a 2010 update. J Hepatol. 2010;52:745–58.

Rubin E, Schaffner F, Popper H. Primary biliary cirrhosis. Chronic non-suppurative destructive cholangitis. Am J Pathol. 1965;46:387–407.

Walker JG, Doniach D, Roitt IM, Sherlock S. Serological tests in diagnosis of primary biliary cirrhosis. Lancet. 1965;1:827–31.

Primary Central Nervous System Vasculitis

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Synonyms

Primary CNS vasculitis: Primary angiitis of the CNS

Definitions

Primary CNS vasculitis: Vasculitis of the blood vessels of the brain and/or spinal cord of unknown cause.

Introduction

Vasculitis of the central nervous system (CNS) may arise from primary involvement of blood vessels in the brain or spinal cord (primary CNS vasculitis), or from secondary involvement of the CNS by a systemic disorder such as systemic vasculitis, connective tissue disease, infection, or malignancy (Hajj-Ali and Calabrese 2012). CNS vasculitis is a rare condition of unknown cause that can ultimately lead to serious neurologic complications including stroke, cognitive impairment, and even death.

The diagnosis of primary CNS vasculitis is a great challenge for physicians (Hajj-Ali et al. 2011; Salvarani et al. 2012). First, it is quite rare. Second, the condition presents with nonspecific symptoms that may mimic conditions that are much more common such as stroke or multiple sclerosis. Clinicians caring for patients with suspected primary CNS vasculitis should be

familiar with its mimics to avoid misdiagnosis. In addition, there are no highly efficient, specific, non-invasive diagnostic modalities for this condition.

Definition

Primary CNS vasculitis is an inflammatory disease that affects vessels in the brain parenchyma, spinal cord, and leptomeninges without evidence of angiitis in other organs.

Epidemiology

Primary CNS vasculitis was first described by Cravioto and Feigin in 1959 in a small series of patients with a chronic, progressive, and fatal form of CNS vasculitis with pathologic findings of granulomatous vasculitis of the brain and spinal cord (Cravioto and Feigin 1959). The estimated annual incidence of primary CNS vasculitis is 2,4 cases/1,000,000, most commonly affecting middle-aged people (40–60 years), especially males (Salvarani et al. 2007).

Clinical and Pathologic Subsets

Recently, there has been an effort to better categorize patients with primary CNS vasculitis into distinct subtypes. This classification helps the clinician to better diagnose, monitor, and treat patients with primary CNS vasculitis (Hajj-Ali et al. 2011).

Granulomatous Angiitis of CNS (GACNS)

GACNS is considered the prototype of primary CNS vasculitis; it is characterized by granulomatous angiitis of predominantly small arteries confined to the brain (Hajj-Ali et al. 2011; Salvarani et al. 2012). It presents with chronic, insidious headache along with diffuse and focal neurologic deficits. Magnetic resonance imaging (MRI) of the brain is almost always abnormal showing nonspecific findings such as high-intensity lesions in the white (T2-FLAIR sequences), deep grey, and subcortical white matter, which tend to be bilateral. Cerebral angiography is not a sensitive test for detecting the small vessel vasculitis of GACNS because of its poor spatial resolution. Analysis of cerebrospinal fluid (CSF) usually reveals an aseptic meningitis profile (i.e., sterile fluid with a lymphocytic pleocytosis and elevated protein).

Cerebral Amyloid Beta-Related Angiitis

The deposition of amyloid beta (Aβ) protein in small and medium-sized vessels in the brain and leptomeninges can cause a number of inflammatory and noninflammmatory vasculopathies referred as cerebral amyloid angiopathy (CAA) and Aβ-related angiitis leading to intracerebral hemorrhages or mass-like lesions. Clinically, patients present with mental status changes, psychotic symptoms (such as hallucinations), and less commonly with focal neurologic deficits. MRI findings are nonspecific showing hyperintense white matter or mass-like lesions. The diagnosis can only be confirmed by brain biopsy showing the characteristic amyloid deposits.

Mass-Like Primary CNS Vasculitis

A subset of patients with CNS vasculitis can present with a solitary brain mass that creates diagnostic confusion with other diseases, especially neoplastic, lymphoproliferative, or infectious (Molloy et al. 2008). Brain biopsy with appropriate additional studies to exclude these diseases (including cultures; specific stains for bacteria, fungi, and mycobacteria; and immunohistochemistry) is imperative in order to make the diagnosis of this rare subtype of CNS vasculitis.

Atypical CNS Vasculitis

The most common category of primary CNS vasculitis is atypical CNS vasculitis (Hajj-Ali et al. 2011; Salvarani et al. 2012). This category

includes cases of biopsy-proven lymphocytic vasculitis (without granulomas) and patients with abnormal imaging (cerebral angiography, MRI) and CSF studies suggestive of CNS vasculitis. In such patients, a meticulous work-up is needed in order to exclude infectious and most importantly lymphoproliferative diseases.

Clinical Manifestations

Vessel inflammation with its resulting ischemia can affect any part of the CNS, causing a number of highly variable and nonspecific clinical manifestations. The most characteristic symptom is an insidious onset chronic headache accompanied in most cases by cognitive changes (Hajj-Ali et al. 2011; Salvarani et al. 2012). Recurrent strokes or transient ischemic attacks can also occur during the course of the disease (30–50 %) and should raise the suspicion for primary CNS vasculitis, especially in young patients when other common causes of strokes have been ruled out (such as cardiovascular disease or a hypercoagulable state). Less common presentations include seizures, myelopathy, and ataxia (Hajj-Ali et al. 2011; Salvarani et al. 2012). Typically, patients with primary CNS vasculitis lack systemic signs or symptoms of vasculitis such as fever, purpura, peripheral neuropathy, or weight loss.

For the diagnosis of the disease, specific simple criteria have been proposed by Calabrese et al. (Table 1) (Calabrese and Mallek 1988).

Diagnostic Assessments

Various laboratory tests of the peripheral blood or CSF, neuroimaging studies (MRI), cerebral angiography, and brain biopsy are included in the evaluation of patients with possible primary CNS vasculitis. However, other than biopsy, there is no specific test for the diagnosis of primary CNS vasculitis. Results of the various diagnostic tests should be interpreted in conjunction with each other by physicians with experience in the diagnosis and treatment of this rare disease.

Primary Central Nervous System Vasculitis, Table 1 Diagnostic criteria for primary vasculitis of the central nervous system

The presence of an acquired and otherwise unexplained neurological or psychiatric deficit
The presence of either classic angiographic or histopathological features of angiitis within the CNS
No evidence of systemic vasculitis or any disorder that could cause or mimic the angiographic or pathological features of the disease

Patients should meet all 3 criteria for the diagnosis of primary CNS vasculitis (Calabrese and Mallek 1988)

Laboratory Tests

Nonspecific markers of systemic inflammation such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are usually normal in disease confined to the CNS such as primary CNS vasculitis (Hajj-Ali et al. 2011; Salvarani et al. 2012). Various serological tests for autoimmune diseases or diseases associated with CNS thrombosis (such as the antiphospholipid syndrome) are required to exclude them. This may include antinuclear antibodies (ANA), antineutrophilic cytoplasmic antibodies (ANCA), and antiphospholipid antibodies. Similarly, an extensive work-up is needed for the exclusion of infectious causes of CNS involvement including cultures (peripheral blood, CSF) and serological or nucleic acid (RNA, DNA) testing. The most important infectious organisms to consider include *Treponema pallidum* (syphilis), *Borrelia Burgdorferi* (neuroborreliosis), *Mycobacterium tuberculosis* (tuberculosis), herpes viruses (e.g., herpes zoster), human immunodeficiency virus (HIV), and various fungi (especially in immunosuppressed individuals) (see Table 2).

CSF analysis is an integral part of the diagnostic work-up of patients with possible primary CNS vasculitis. Although there are no specific abnormalities of the CSF, it is of great importance to obtain cultures, microbiologic stains and cultures, cytology and flow cytometry in order to rule out infectious or malignant processes. In approximately 80–90 % of patients with pathologically documented disease, CSF analysis is abnormal with elevated protein levels and modest lymphocytic pleocytosis.

Primary Central Nervous System Vasculitis, Table 2 Common diseases that may mimic primary CNS vasculitis

A. Secondary CNS vasculitides
1. *Infectious*
Viral (HIV, herpes zoster)
Bacterial (syphilis, tuberculosis, neuroborelliosis)
F*ungal (aspergillosis)*
2. *Rheumatologic disorders*
Behçet's disease
ANCA-associated vasculitides (Granulomatosis with polyangiitis, Churg-Strauss syndrome)
Systemic lupus erythematosus
Sjögren's syndrome
3. *Neoplastic disorders*
Lymphomas (Hodgkin and Non-Hodgkin)
Leukemia
Lung cancer
Angiocentric lymphoproliferative disorders
B. Non-vasculitic disorders
Reversible cerebral vasoconstriction syndromes (RCVS)
Antiphospholipid syndrome and other hypercoagulable disorders
Atherosclerosis
Embolic diseases
Miscellaneous (e.g., sarcoidosis or genetic diseases, such as Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL))

Imaging Studies

Brain MRI

The vast majority of patients with primary CNS vasculitis have abnormal MRI (90–100 %) which makes it an important study to perform early in the evaluation. A normal MRI is a strong argument against the diagnosis of CNS vasculitis (Hajj-Ali et al. 2011; Salvarani et al. 2012). Typical MRI findings include multiple infarcts in different vascular territories, nonspecific high-intensity white matter lesions, and, less frequently, leptomeningeal enhancement or mass-like lesions. MRI has a greater sensitivity than CT and is the preferred initial imaging modality.

Angiography

Imaging of the CNS vasculature either by invasive angiography or CT/MR-angiography can reveal a number of lumenal abnormalities in visualized vessels including the characteristic stenosis followed by dilated lesions ("beading") in one or (more characteristically) multiple vessels. Despite the significant advances in imaging techniques, angiography still has a rather poor sensitivity (~60 %), primarily due to the small size of affected vessels (<300 μm) which is lower than the minimal size that the angiography can detect (>500 μm). Furthermore, "vasculitis-like" lesions can be seen in a number of non-vasculitic disorders such as vasospasm, atherosclerosis, and infection. This limits the specificity of the test for the diagnosis of primary CNS vasculitis to approximately 30 %.

Brain Biopsy

Brain and leptomeningeal biopsy remains the gold standard for the diagnosis of primary CNS vasculitis and most importantly for the exclusion of "vasculitis-mimics" (such as infectious or malignant diseases). Despite concerns about potential neurological complications, brain biopsy has a low risk (1 %) when performed in experienced centers (Salvarani et al. 2012).

Typical findings include granulomatous, necrotizing, or lymphocytic transmural inflammation of small- or medium-sized arteries. Given the focal and segmental distribution of the involved lesions, the sensitivity of the biopsy ranges from 53 % to 63 %. Nevertheless, directed biopsy of a radiographically abnormal or suspicious lesion can increase its sensitivity to 78 % (Salvarani et al. 2012). Collectively, these data emphasize the importance of a directed biopsy in the diagnostic work-up of patients with suspected primary CNS vasculitis. But, it is equally essential to recognize that a number of patients can still have primary CNS vasculitis with a negative brain biopsy.

Differential Diagnosis

Numerous diseases that affect the CNS and have similar clinical, angiographic, imaging,

or histological appearance to primary CNS vasculitis can create diagnostic confusion (see Table 2).

Among them, the group of reversible cerebral vasoconstriction syndromes (RCVS) is frequently mistaken as primary CNS vasculitis (Calabrese et al. 2007). This group is characterized by prolonged but reversible vasoconstriction of the cerebral arteries and includes Call-Fleming syndrome, drug-induced angiopathy (especially due to cocaine), migraine, benign angiopathy of the central nervous system, pheochromocytoma, poorly controlled or malignant hypertension, postpartum angiopathy, and drug-induced vasospasm. RCVS usually presents with sudden onset thunderclap type of headache accompanied in almost half of the cases by focal neurologic deficits or seizures (Calabrese et al. 2007). Angiography can show a picture identical to that seen in primary CNS vasculitis with beading of involved arteries, while MRI can reveal infarcts, hemorrhagic (cortical or subarachnoid), and edematous lesions (Calabrese et al. 2007). CSF analysis is usually normal. The clinical course of RCVS is benign with reversal of the vasoconstriction in the first 3 months after presentation.

A diverse number of infectious, autoimmune, neoplastic, or noninflammatory disorders can cause either a secondary CNS vasculitis or mimic CNS vasculitis (Table 2). As mentioned earlier, a thorough diagnostic work-up utilizing appropriate diagnostic modalities in tertiary centers by experienced subspecialists (such as rheumatologists, radiologists, neurologists, infectious disease specialists, and/or pathologists) are needed in order to exclude these diseases and make the appropriate diagnosis.

Treatment

Earlier reports of patients with primary CNS vasculitis have described a dismal prognosis. Over the last few decades, a number of studies have shown that immunosuppressive therapy is associated with a better outcome (Salvarani et al. 2007). Treatment of primary CNS vasculitis remains empirical since no controlled studies have ever been performed.

Therapy is usually initiated by high-dose corticosteroids followed in resistant cases with agents such as cyclophosphamide. The treatment is given until clinical remission is achieved (usually for 3–6 months). Assessing the response to therapy and differentiating between residual active disease and permanent damage is not an easy task. Serial imaging studies may be warranted. Once patients have reached clinical remission or stabilization of their condition, maintenance therapy with azathioprine or mycophenolate mofetil is started. Using this therapeutic approach, an initial response to treatment is observed in approximately 80 % of cases; relapses occur in approximately one-quarter of the patients (Salvarani et al. 2007). The 5- and 10-year survival rate of patients with primary CNS vasculitis is decreased compared to the general population (~60 %), indicating the need for novel therapies for this disease (Salvarani et al. 2007).

Cross-References

- ▶ Cancer and the Central Nervous System
- ▶ Vasculitis: Granulomatosis with Polyangiitis (Wegener's)

References

Calabrese LH, Mallek JA. Primary angiitis of the central nervous system. Report of 8 new cases, review of the literature, and proposal for diagnostic criteria. Med (Baltimore). 1988;67:20–39.

Calabrese LH, Dodick DW, Schwedt TJ, Singhal AB. Narrative review: reversible cerebral vasoconstriction syndromes. Ann Intern Med. 2007;146:34–44.

Cravioto H, Feigin I. Noninfectious granulomatous angiitis with a predilection for the nervous system. Neurol. 1959;9:599–609.

Hajj-Ali RA, Calabrese LH. Primary central nervous system vasculitis. In: Hoffman GS, Weyand CM, Langford CA, Goronzy JJ, editors. Inflammatory diseases of blood vessels. Chichester: Wiley-Blackwell; 2012. p. 322–31.

Hajj-Ali RA, Singhal AB, Benseler S, Molloy E, Calabrese LH. Primary angiitis of the CNS. Lancet Neurol. 2011;10:561–72.

Molloy ES, Singhal AB, Calabrese LH. Tumour-like mass lesion: an under-recognised presentation of primary angiitis of the central nervous system. Ann Rheum Dis. 2008;67:1732–5.

Salvarani C, Brown Jr RD, Calamia KT, Christianson TJ, Weigand SD, Miller DV, Giannini C, Meschia JF, Huston III J, Hunder GG. Primary central nervous system vasculitis: analysis of 101 patients. Ann Neurol. 2007;62:442–51.
Salvarani C, Brown Jr RD, Hunder GG. Adult primary central nervous system vasculitis. Lancet. 2012;380:767–77.

Primary Sclerosing Cholangitis: Clinical and Systemic Manifestations and Treatment

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Definition

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by progressive inflammation, fibrosis, and destruction of the intrahepatic and extrahepatic bile ducts, eventually resulting in biliary cirrhosis and end-stage liver disease (Chapman et al. 2010).

Clinical Manifestations

Patients with primary sclerosing cholangitis generally present with abnormally elevated liver tests, specifically an elevated alkaline phosphatase level. Common symptoms include fatigue and pruritus, as well as jaundice in patients with advanced disease. Fever, chills, and right upper quadrant pain may arise in patients with concurrent bacterial cholangitis.

PSC is diagnosed by establishing specific features of the disease via cholangiography after exclusion of secondary causes such as bacterial cholangitis, autoimmune pancreatitis, cholangiopathies, and malignancies (Berstad et al. 2006). The most important feature of PSC is the stricturing and dilation of both intrahepatic and extrahepatic bile ducts. Isolated stricturing of the extrahepatic or intrahepatic bile ducts may also be present. Patients with early-stage disease may have only shallow ulcerations of the bile ducts, and stricturing can develop as the disease progresses. Presence of stricturing is most commonly observed via magnetic resonance cholangiography. Endoscopic resonance cholangiography was once the standard course of action, but it is being used less frequently in diagnosis, as it leads to complications requiring hospitalization in over 10 % of PSC patients who undergo the procedure (Bangarulingam et al. 2009). Liver biopsy is not required in patients with characteristic cholangiographic findings but is advised in patients with suspected small-duct PSC and normal cholangiographies (Dave et al. 2010). Histologic features of PSC have low specificity. The most specific finding is destruction of the bile ducts and substitution thereof by connective tissue, which may extend into the liver parenchyma as the disease advances (Ludwig et al. 1981). Histologic findings allow staging of PSC as shown in Table 1.

Primary Sclerosing Cholangitis: Clinical and Systemic Manifestations and Treatment, Table 1 Staging of PSC

PSC stages	Finding
Stage 1	Inflammation of the portal triads with mononuclear infiltration and piecemeal necrosis
Stage 2	Fibrosis expanding into the parenchyma with dilation of the portal triads
Stage 3	Bridging fibrosis
Stage 4	Cirrhosis

Liver biochemistries may fluctuate in patients with primary sclerosing cholangitis, although elevation of alkaline phosphatase is usually the predominant pattern. Aminotransferases are moderately elevated and albumin levels may be decreased, especially in patients with inflammatory bowel disease. Altered prothrombin time and albumin level in a patient's serum reflect disease progression (Wiesner et al. 1989). Patients may also have elevated levels of gamma globulins, serum immunoglobulin M (IgM), and serum

immunoglobulin G4 (IgG4) levels (Mendes et al. 2006). Autoantibodies present in patients with primary sclerosing cholangitis include antismooth, antinuclear, and anticardiolipin antibodies. Anticardiolipin antibodies correlate with Mayo risk score, whereas other antibodies are of little prognostic significance (Angulo et al. 2000).

Systemic Complications

Primary sclerosing cholangitis has a strong association with inflammatory bowel disease (IBD). The prevalence of IBD in PSC has been reported as 60–80 % (Chapman et al. 2010). Patients with primary sclerosing cholangitis and inflammatory bowel disease are also at an increased risk of colonic neoplasia (Soetikno et al. 2002).

The most dreaded complication of PSC is cholangiocarcinoma (CCA), with an annual incidence of around 0.5–1.5 % and a lifetime risk of 10–15 % in PSC patients (Fevery et al. 2007). The presence of CCA denotes a poor prognosis. In one report, only 10 % of patients survived during the first 2 years of follow-up (Wiesner 1994). This high rate of morality is in part due to the difficulty of diagnosis of CCA. Presentation is usually nonspecific and highly variable, and hence the disease is often already at an advanced tumor stage by the time it is diagnosed. Patients developing CCA may present with weight loss, jaundice, abdominal discomfort, and quick clinical deterioration which can lead to its discovery. Diagnosis of cholangiocarcinoma in patients with PSC is established via some combination of ultrasound computed tomography, magnetic resonance imaging, brush cytology, endobiliary biopsy, and serum carbohydrate antigen 19–9 (CA 19–9) level (Rosen et al. 1991). Diagnosis via visual methods alone is unreliable as the appearance of CCA is similar to that of biliary strictures common in PSC patients. Additionally, there have historically been no clinically useful predictive factors for CCA, although several have been suspected, including alcohol consumption, presence of dominant strictures, variceal bleeding, cirrhosis, and presence of inflammatory bowel disease (Wiesner 1994). Screening criteria for CCA are being developed and are discussed later in this entry.

There are several other complications associated with PSC. One such complication is the development of dominant biliary strictures. Dominant biliary strictures develop in 45–58 % of PSC patients and may occur anywhere along the common hepatic or common bile duct (Chapman et al. 2010). Exclusion of malignancy is essential upon identifying a dominant stricture, as they are difficult to distinguish from cholangiocarcinoma. This is done by endoscopic biopsy and/or brush cytology (Chapman et al. 2010). Another complication of PSC is bacterial cholangitis. Patients with PSC may develop bacterial cholangitis spontaneously, especially if they have bile duct stones or strictures. The risk is increased in patients undergoing a liver biopsy or endoscopic and surgical manipulation (Porayko et al. 1991).

Other complications include hepatic osteodystrophy (osteopenia or osteoporosis), portal hypertension (PHT), and gallbladder disease. Hepatic osteodystrophy is associated with many chronic liver disorders and commonly develops in patients with advanced PSC. Risk factors for these conditions include age greater than 54 years, body mass index of 24 kg/m^2 or less, and presence of inflammatory bowel disease for 19 years or more (Angulo et al. 2011). PHT gradually develops in PSC patients with cirrhosis, as it does in all patients with cirrhosis. Finally, 30 % of patients with PSC may develop cholelithiasis and choledocolithiasis due to cholesterol or pigment stones (Mack 1995).

Treatment of Primary Sclerosing Cholangitis

There is currently no proven treatment which slows the progression of primary sclerosing cholangitis. A variety of anti-inflammatory and immunosuppressive agents, among others, have been tested, but with limited success (Poropat et al. 2011). Thus, the primary focus of treatment is on management of symptoms and

complications associated with PSC. This includes regular screening for cancers which commonly arise in the setting of PSC. For patients who have progressed to advanced disease, good outcomes can be achieved with liver transplantation (Graziadei et al. 1999). An overview of management of progressive disease is presented below.

Drug Therapy with Ursodeoxycholic Acid

The most extensively studied treatment for PSC is ursodeoxycholic acid (UDCA), a hydrophilic dihydroxy bile acid. UDCA is generally well tolerated and currently used as an effective treatment for primary biliary cirrhosis (PBC), a cholestatic liver disorder with some similarities to PSC (Lindor et al. 2009). Unfortunately, although UDCA has been shown to significantly improve liver biochemistries in PSC patients, it appears to have no proven beneficial effect on clinical endpoints, such as death or need for liver transplantation. It also has no significant effect on liver histology or clinical symptoms, including fatigue and pruritus (Poropat et al. 2011). High doses (28–30 mg/kg/day) are associated with worse clinical outcomes compared to placebo, especially in patients with early-stage disease and normal serum bilirubin levels. Nevertheless, UDCA is still prescribed for PSC patients due to its historical importance, as well as suspicions that the dosage and/or timing of treatment may play a role in the progression of PSC. There have been several studies to this end, but the full picture is only beginning to emerge.

Management of Symptoms and Complications

As there is no treatment for PSC, the primary goal of care is managing associated symptoms and complications. One common symptom is pruritus. Bile acid sequestrants, such as cholestyramine, are generally the treatment of choice. Similar to managing pruritus in patients with primary biliary cirrhosis, rifampicin or oral opiate antagonists may be prescribed if cholestyramine fails to relieve symptoms (Lindor et al. 2009; Chapman et al. 2010).

Pruritus can be perpetrated by dominant strictures, which can lead to other symptoms (e.g., cholangitis, jaundice) and impaired liver function. Existence of such strictures should be considered before beginning antipruritic drug therapy, particularly in patients with a worsening clinical presentation. As for treatment, various endoscopic and percutaneous approaches are available. The most common is usage of endoscopic retrograde cholangiopancreatography (ERCP) to further investigate and treat strictures with endoscopic techniques, primarily balloon dilation. However, it is recommended to perform brush cytology and/or endoscopic biopsy to exclude cholangiocarcinoma, which has an appearance very similar to benign biliary strictures, while pursuing endoscopic therapy. Occasionally, neither treatment of dominant strictures nor drug therapy is effective in alleviating a patient's pruritus. In these cases, liver transplantation may be indicated (Chapman et al. 2010). Bacterial cholangitis can also arise from dominant strictures. Specifically, biliary obstruction can lead to bacterial colonization of bile, resulting in secondary cholangitis. In general, patients respond well to drainage of the obstruction, plus antibiotics. For patients with recurrent bacterial cholangitis, the American Association for the Study of Liver Diseases (AASLD) recommends the use of prophylactic long-term antibiotics. As with pruritus, severe bacterial cholangitis may also be an indication for liver transplant (Chapman et al. 2010).

Other notable complications of PSC include the aforementioned hepatic osteodystrophy and portal hypertension. For the former, the AASLD recommends bone density examinations at diagnosis of PSC and at 2–3-year intervals afterwards. Treatment for PSC patients with osteopenia or osteoporosis includes calcium and vitamin D supplements and occasionally bisphosphonates as well depending on disease severity. As for PHT, treatment in patients with PSC is the same as for those without PSC.

This entails medications, endoscopic therapy, surgery, and radiologic procedures (Chapman et al. 2010).

Associated Malignancies and Screening

Primary sclerosing cholangitis is considered a premalignant condition, as patients with PSC have an increased risk of hepatic and extrahepatic malignancies. As discussed previously, the most grave of these is cholangiocarcinoma. Screening criteria for CCA and the optimal selection of patients for screening are yet to be determined. One suggestion is that all PSC patients undergo annual ultrasound or magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreatography (MRCP), plus CA 19–9 determination (Razumilava et al. 2011). Regardless of the ongoing debate over screening and surveillance for CCA, the AASLD recommends that PSC patients who experience rapid clinical deterioration, such as weight loss or development of jaundice, should be evaluated for CCA (Chapman et al. 2010).

Due to the difficulty of diagnosis, cholangiocarcinoma is typically diagnosed at an advanced tumor stage, significantly limiting treatment options. Those who are diagnosed with advanced disease generally can only be given palliative care. Potentially curative resection is only an option for patients without cirrhosis, multifocal disease, or other advanced presentation, but even for such patients, the 3-year survival rate is less than 20 % (Chapman et al. 2010). The best outcomes are experienced by patients with early-stage cholangiocarcinoma who successfully undergo liver transplantation preceded by a thorough workup including neoadjuvant therapy. However, this course of action requires careful candidate screening and involves a complicated treatment regimen.

Primary sclerosing cholangitis is also associated with gallbladder cancer (GBC) and gallbladder disease in general. One study found the prevalence of all gallbladder abnormalities in PSC patients to be approximately 40 % (Said et al. 2008). The prevalence of gallbladder mass lesions is 3–14 % (Razumilava et al. 2011). The AASLD recommends annual ultrasound to detect such lesions in the gallbladder (Chapman et al. 2010). The general consensus historically has been to remove any gallbladder lesions in PSC patients regardless of size, but this has been challenged by recent examinations of early and long-term postoperative outcomes, as well as predictors of malignancy in PSC patients with gallbladder polyps. New data suggest that removal via cholecystectomy should be performed for polyps $\geq$0.8 cm, and it may be considered for smaller polyps if the patient's liver function is good. Any small polyps found but not removed should be monitored on a more regular basis, such as every 3–6 months instead of annually (Razumilava et al. 2011).

Yet another cancer known to be associated with PSC is hepatocellular carcinoma (HCC). Nearly all causes of cirrhosis correspond to an increased risk of HCC, and PSC is no exception. The incidence of HCC in PSC patients has not been well studied, however, and may be too low to warrant a separate screening regimen. Annual imaging studies for CCA and GBC surveillance may nonetheless assist in the detection of HCC as well (Razumilava et al. 2011).

Another malignancy associated with PSC, specifically in PSC patients who also have IBD, is colorectal cancer (CRC). In patients with both PSC and ulcerative colitis (UC), the cumulative incidence of CRC approaches 50 % after 25 years of colitis (Torres et al. 2011). Such patients are at a higher risk of CRC and dysplasia compared to patients with UC alone (Soetikno et al. 2002). Additionally, for patients with IBD, there is on average only a short-time interval between PSC diagnosis and development of CRC. As such, colonoscopy with biopsies at 1- to 2-year intervals is strongly recommended for patients with confirmed PSC and IBD (Graziadei et al. 1999; Soetikno et al. 2002; Chapman et al. 2010). For patients with PSC but no previous history of IBD, a full colonoscopy with biopsies is recommended upon PSC diagnosis. In most cases, however, IBD diagnosis precedes PSC diagnosis. Regarding management of IBD and

CRC, treatment of PSC patients is largely the same as treatment of non-PSC patients (Chapman et al. 2010).

Liver Transplantation

In the long term, the only life-prolonging treatment for primary sclerosing cholangitis is liver transplantation. Indications for transplantation for patients with PSC are similar to those for other chronic liver diseases; chronic liver failure and significantly reduced quality of life are two examples. Indications particular to PSC include intractable pruritus, recurrent bacterial cholangitis, and, in some cases, cholangiocarcinoma. As discussed previously, patients with early-stage cholangiocarcinoma can experience good outcomes with liver transplant, provided they are carefully selected and undergo neoadjuvant therapy beforehand (Chapman et al. 2010). Those with more advanced disease are not viable candidates for transplant, as cholangiocarcinoma has been shown to recur quickly in the vast majority of these cases (Graziadei et al. 1999).

In general, liver transplantation for PSC is successful, with roughly 85 % of patients surviving at least 5 years posttransplant. Disease recurrence is possible, however, and occurs in approximately one fifth to one fourth of patients within 5–10 years (Graziadei et al. 1999).

Conclusion

Primary sclerosing cholangitis is a chronic cholestatic liver disease that presents via elevated liver tests and eventually progresses to end-stage liver disease. PSC is commonly accompanied by IBD and is associated with several short- and long-term complications. The mainstay of treatment remains obscure, with no current treatment, other than liver transplantation, that improves outcomes, despite the positive effect of UDCA on improving liver biochemistries. Timely management of complications and the institution of recommended screening may prove beneficial in improving long-term outcomes for PSC patients.

Cross-References

- ▶ Fibrosis
- ▶ Impact of Recurrent Autoimmune Diseases in Renal Transplant Outcomes
- ▶ Primary Biliary Cirrhosis, Overview

References

Angulo P, Peter JB, Gershwin ME, DeSotel CK, Shoenfeld Y, Ahmed AE, Lindor KD. Serum autoantibodies in patients with primary sclerosing cholangitis. J Hepatol. 2000;32(2):182–7.

Angulo P, Grandison GA, Fong DG, Keach JC, Lindor KD, Bjornsson E, Koch A. Bone disease in patients with primary sclerosing cholangitis. Gastroenterology. 2011;140(1):180–8.

Bangarulingam SY, Gossard AA, Petersen BT, Ott BJ, Lindor KD. Complications of endoscopic retrograde cholangiopancreatography in primary sclerosing cholangitis. Am J Gastroenterol. 2009;104(4):855–60.

Berstad AE, Aabakken L, Smith HJ, Aasen S, Boberg KM, Schrumpf E. Diagnostic accuracy of magnetic resonance and endoscopic retrograde cholangiography in primary sclerosing cholangitis. Clin Gastroenterol Hepatol. 2006;4(4):514–20.

Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. Hepatology. 2010;51(2):660–78.

Dave M, Elmunzer BJ, Dwamena BA, Higgins PD. Primary sclerosing cholangitis: meta-analysis of diagnostic performance of MR cholangiopancreatography. Radiology. 2010;256(2):387–96.

Fevery J, Verslype C, Lai G, Aerts R, Van Steenbergen W. Incidence, diagnosis, and therapy of cholangiocarcinoma in patients with primary sclerosing cholangitis. Dig Dis Sci. 2007;52(11):3123–35.

Graziadei IW, Wiesner RH, Marotta PJ, Porayko MK, Hay JE, Charlton MR, Poterucha JJ, Rosen CB, Gores GJ, LaRusso NF, Krom RA. Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. Hepatology. 1999;30(5):1121–7.

Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. Hepatology. 2009;50(1):291–308.

Ludwig J, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hepatology. 1981;1(6):632–40.

Mack E. Pathogenesis and clinical presentation of bile duct calculi. Semin Laparosc Surg. 1995;2(2):76–84.

Mendes FD, Jorgensen R, Keach J, Katzmann JA, Smyrk T, Donlinger J, Chari S, Lindor KD. Elevated

serum IgG4 concentration in patients with primary sclerosing cholangitis. Am J Gastroenterol. 2006; 101(9):2070–5.
Porayko MK, LaRusso NF, Wiesner RH. Primary sclerosing cholangitis: a progressive disease? Semin Liver Dis. 1991;11(1):18–25.
Poropat G, Giljaca V, Stimac D, Gluud C. Bile acids for primary sclerosing cholangitis. Cochrane Database Syst Rev. 2011;1:CD003626.
Razumilava N, Gores GJ, Lindor KD. Cancer surveillance in patients with primary sclerosing cholangitis. Hepatology. 2011;54(5):1842–52.
Rosen CB, Nagorney DM, Wiesner RH, Coffey Jr RJ, LaRusso NF. Cholangiocarcinoma complicating primary sclerosing cholangitis. Ann Surg. 1991;213(1):21–5.
Said K, Glaumann H, Bergquist A. Gallbladder disease in patients with primary sclerosing cholangitis. J Hepatol. 2008;48(4):598–605.
Soetikno RM, Lin OS, Heidenreich PA, Young HS, Blackstone MO. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis: a meta-analysis. Gastrointest Endosc. 2002;56(1):48–54.
Torres J, de Chambrun GP, Itzkowitz S, Sachar DB, Colombel JF. Review article: colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease. Aliment Pharmacol Ther. 2011;34(5):497–508.
Wiesner RH. Current concepts in primary sclerosing cholangitis. Mayo Clin Proc. 1994;69(10):969–82.
Wiesner RH, Grambsch PM, Dickson ER, Ludwig J, MacCarty RL, Hunter EB, Fleming TR, Fisher LD, Beaver SJ, LaRusso NF. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology. 1989;10(4):430–6.

Primary T-Cell Activation in Liver

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Synonyms

Intrahepatic T-cell activation

Definition

Naïve T cells are normally activated in secondary lymphoid organs (lymph nodes and spleen) by professional antigen-presenting cells. The liver is the only nonlymphoid compartment where primary T-cell activation occurs. This intrahepatic activation is generally mediated by nonprofessional antigen-presenting cells and is thus governed by different rules than those existing in lymphoid tissues. Understanding the parameters influencing intrahepatic T-cell activation is thus critical to understanding how the liver influences immune responses to hepatic pathogens.

Introduction

Lymphocytes circulating via the blood are not generally able to directly contact parenchymal cells in most tissues. Endothelial cells and the tissue basal membrane form an effective physical barrier that excludes any contact between the two cell types. Thus, to access tissues and to overcome the strong shear force created by blood flow, lymphocytes need to express a range of molecules that promote strong adhesion, migration, and degradation of plasma membrane proteins. Due to its unique architecture and pattern of blood follow (vide infra), the liver is an exception to this rule of lymphocyte trafficking, allowing different liver cells to establish direct contact with blood lymphocytes and, if they express the relevant peptide/major histocompatibility complexes (p/MHC), to induce activation of T cells. The physiological role, significance, and consequence of this liver-specific direct T-cell activation are still the subject of current research, but there is no doubt that this alternative pathway of activation would have a deep influence on the immune response to liver-specific pathogens and to liver-expressed self-antigens during autoimmunity. This entry will discuss the evidence for direct T-cell activation in the liver, the characteristics of different hepatic antigen-presenting cells (APC), and the fate of these T cells.

The Liver Influences T-Cell Circulation

T cells develop in the thymus and recirculate via the blood and the lymph in an immature state. These resting or naïve T cells lack many molecules expressed by effector cells, including those that are required to adhere to the endothelium of most capillaries. However, they do express L-selectin, a molecule that facilitates adhesion to the high endothelial venules of the lymph nodes. Entry into the lymph nodes allows naïve T cells to scan for foreign antigens presented by dendritic cells (DCs) in the form of p/MHC. Primary T-cell activation by DCs allows blast formation, cytokine secretion, proliferation, and differentiation into cells endowed with effector function and the capacity for trans-endothelial migration. The ability of naïve T cells to be activated solely in lymphoid tissues is an immunological paradigm that has been experimentally proven in many organs but with one exception: the liver.

The most convincing evidence of this hepatic property comes from studies using transgenic mouse models: naïve T-cell receptor (TCR) transgenic $CD8^+$ T cells adoptively transferred into transgenic mice with expression of the cognate p/MHC restricted to the liver were retained within 1 h of transfer in this organ and underwent activation in situ (Bertolino et al. 2001; Bowen et al. 2004). No sign of activation was observed in lymphoid organs or other tissues, demonstrating that the liver is a site of primary activation independent of lymphoid tissues. More recently in a murine model, primary T-cell activation was also observed in liver transplants in the absence of extrahepatic T-cell activation in the recipients (Klein and Crispe 2006).

Primary T-cell activation in a solid organ is unique to the liver: when the same transgenic radiolabelled naïve T cells were adoptively transferred into transgenic mice ubiquitously expressing the cognate p/MHC, the liver was the only organ that retained the donor cells at 1 h post-adoptive transfer in an antigen-specific manner (Bowen et al. 2002; Bertolino et al. 2005). Lymphoid organs contained a much lower proportion of transferred radioactivity at this early time point, suggesting that the liver and secondary lymphoid tissues are the only sites contributing and competing for primary T-cell activation (Bowen et al. 2002; Bertolino et al. 2005). This intrahepatic retention has been shown to be mostly dependent on p-MHC/TCR and ICAM-1/LFA-1 interactions (Bertolino et al. 2005).

The liver also affects the circulation of activated/effector CD4 and CD8 T cells. When anti-hepatitis B surface antigen (anti-HBsAg) polyclonal CTL lines were adoptively transferred into transgenic mice ubiquitously expressing HBsAg, infiltration and tissue damage occurred only within the liver, despite similar antigen expression in other sites. Cytopathic damage to renal tubules in the kidney and choroid plexus epithelial cells in the brain did not occur unless CTL were injected beneath the kidney capsule or intracerebrally (Ando et al. 1994). Unlike naïve T cells that are only intrahepatically retained upon recognition of their cognate antigen, retention of effector cells in the liver is antigen independent. ICAM-1/LFA-1 interactions have been shown to play a key role in this adhesion (Mehal et al. 1999). It is likely that this "retention" reflects a slow transit of effector cells in the narrow sinusoids, rather than cells establishing permanent residency in the organ (Klugewitz et al. 2004).

The role of the liver in supporting primary T-cell activation has only been shown for CD8 T cells so far, due to the difficulty of restricting MHC class II expression and antigen to MHC class II liver APCs. Whether naïve CD4 T cells can be activated in the liver is still a matter of debate.

The Hepatic Sinusoids

The structure and cellular composition of the liver sinusoids (▶ Ultrastructure of the Liver Sinusoid) are key to understand its role in intrahepatic T-cell retention. These capillaries have an average diameter of about 10 μm and are lined by endothelial cells. Due to the narrow size of the sinusoids, interactions between T lymphocytes and liver endothelial cells occur under conditions of low velocity blood flow,

a property that is important in favoring leukocyte recruitment within the liver, which has been shown to occur in the absence of selectins (Wong et al. 1997; Bowen et al. 2004). Flow of arterial blood in the sinusoids is also intermittent due to the contraction of contractile smooth muscle sphincters in the walls of hepatic arterioles (MacPhee et al. 1995; MacSween and Scothorne 1979). Kupffer cells patrolling the sinusoids also obstruct the lumen of these capillaries and contribute to intermittent blood flow (McCuskey and Reilly 1993). Liver sinusoidal endothelial cells (LSEC) possess unusual properties; unlike other endothelial cells, they do not form tight junctions with adjacent endothelial cells but form an incomplete barrier between the sinusoidal lumen and the perivascular parenchymal tissue. Furthermore, these cells are perforated by several holes, or fenestrae, up to 120 nm in diameter (Wisse et al. 1985). These fenestrae are grouped in clusters forming sieve plates and behave as dynamic structures that dilate or contract to facilitate solute exchange between the plasma and hepatocytes (Wisse et al. 1985). Both hepatocytes and stellate cells are located under the endothelial cell layer. The liver also contains a significant number of immature dendritic cells, consistent with frequencies in other tissues. Although some DCs have been shown to transit the sinusoids (Matsuno et al. 1996), they appear to reside mostly within the portal tracts.

Antigen-Presenting Cells in the Liver

The liver receives up to 25 % of the cardiac output. Thus, every T cell in the body passes through the liver multiple times during its life and has the opportunity to interact with liver cells in the sinusoids. There has been extensive debate in the literature about the contribution of various liver APCs to intrahepatic T-cell activation. Experimental evidence has demonstrated that they can all act as APCs in vitro (reviewed in Bertolino et al. 2002). In vivo, two parameters would restrict activation: physical access to T cells and antigen expression. KC and LSEC are located in the lumen of the sinusoids and are the most accessible cells to blood lymphocytes. Hepatocytes and stellate cells are located underneath the endothelial barrier and are thus less accessible. However, electron microscopy micrographs have demonstrated that hepatocytes establish contact with leukocytes through LSEC fenestrations (Warren et al. 2006). Although not experimentally demonstrated, it is likely that similar contacts occur between stellate cells and leukocytes. There is no data in the literature indicating that DCs and BECs can induce primary T-cell activation within the liver. Whether liver DCs interact with circulating T lymphocytes has not been shown; however, if most DCs reside in the portal tracts as suggested by the literature, they would not be accessible to naïve T cells. Likewise, biliary epithelial cells (BECs) are located in portal tracts and are unlikely to play an important role in primary T-cell activation. Consistent with the above, the main liver cell types shown to play a role in primary activation are LSEC, KC, stellate cells, and hepatocytes, and their role will be briefly reviewed below.

Kupffer cells (▶ Kupffer Cells in Immune Tolerance) rest on LSEC and occasionally within gaps separating adjacent LSEC. Recent studies using intravital spinning disk and multiphoton microscopy have demonstrated that they are able to efficiently capture pathogens circulating in the sinusoids, such as *Borrelia burgdorferi* (Lee et al. 2010), and contribute to the formation hepatic granulomas in *Leishmania donovani* (Beattie et al. 2010) and *Mycobacterium bovis* BCG (Egen et al. 2008) infections. They express MHC class I and II molecules, ICAM-1, CD86, and CD80 and function as APCs for naïve CD8 T cells and CD4 T cells in vitro. In vivo, there is now data showing that liver bone marrow-derived cells (that include both KC and DC) are able to efficiently retain adoptively transferred naïve TCR transgenic CD8 T-cell mice in an antigen-specific manner (Bowen et al. 2002; Holz et al. 2012). This retention leads to the rapid activation of the transgenic T cells in situ. The fate of CD8 T cells activated by KC has not been fully studied, but recent results suggest that in the absence of inflammation, this activation leads to CD8 T-cell deletion (Holz et al. 2012). The role of KC in shaping CD4 and CD8 T-cell

responses is reviewed in a different entry of this encyclopedia (▶ Kupffer Cells in Immune Tolerance).

LSEC (▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance) are scavenger cells strategically located in the sinusoids to enable their extraction of blood-borne material, as demonstrated by their ability to clear low-density lipoprotein (LDL) and capture particulate antigens and immune complexes. This uptake is mediated by mannose receptor, and has been shown to be more efficient than KC, suggesting that these cells specialize in this function. LSEC constitutively express both MHC class I and class II molecules, low levels of CD86, as well as adhesion molecules including ICAM-1, VCAM-1, and dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN). They have been shown to be efficient APCs to both naïve CD4 and CD8 T cells in vitro (Knolle et al. 1999). More importantly, LSEC have also been shown to be capable of cross-presentation of soluble antigens on MHC class I molecules to CD8 T cells (Limmer et al. 2000), a property that is shared only by some subsets of DCs. In addition to their immunological properties, LSEC possess fenestrations that act as a portal between T cells and cells underlying the endothelial layer (hepatocytes and stellate cells) (Warren et al. 2006). The role of LSEC in shaping CD4 and CD8 T-cell responses is reviewed in a different entry of this encyclopedia (▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance).

Hepatocytes are the principal cell of the liver, comprising 75 % of hepatic cells. They are large polyhedral, often binucleated cells involved in the synthesis and secretion of a wide variety of biologically crucial molecules, as well as the metabolism and excretion of an extensive range of both endogenous and xenobiotic substances. In the absence of liver inflammation, hepatocytes express MHC class I molecules, CD1, and ICAM-1; MHC class II molecules, CD40L, and costimulatory molecules such as CD80 and CD86 are not constitutively expressed (Bertolino et al. 1998; Holz et al. 2012), however can be upregulated following inflammation. Since they normally lack MHC class II expression, resting hepatocytes may therefore only act as APCs for CD1 and MHC class I restricted T cells. Primary hepatocytes are able to drive efficient activation and proliferation of TCR transgenic naïve CD8 T cells in vitro (Bertolino et al. 1998). Although separated from leukocytes by an endothelial barrier, several studies have shown that hepatocytes interact with and activate TCR transgenic naïve CD8 T cells circulating via the blood in vivo (Bertolino et al. 2001; Bowen et al. 2004; Derkow et al. 2007; Klein and Crispe 2006). These contacts seem to occur via LSEC fenestrations and to require ICAM-1/LFA-1 interactions. Hepatocytes are not able to cross-present antigen like LSEC (Limmer et al. 2000).

The functional outcome of T-cell activation by hepatocytes remains controversial. In some studies, CD8 T cells activated by hepatocytes proliferated but were deleted within the first days postactivation, without developing significant cytotoxic activity. In those studies, most CD8 T cells were rapidly deleted within the first few hours postactivation as a result of invading hepatocytes and degradation into lysosomal compartments (Benseler et al. 2011). Some T cells surviving this process proliferated but subsequently died as a result of primary activation in the absence of costimulatory molecules. This "death by neglect" was associated with low expression of cytokines and high expression of the proapoptotic molecule Bim (Holz et al. 2008; Holz et al. 2012). In contrast to these studies suggesting that hepatocytes induce T-cell deletion and tolerance, several studies have suggested that CD8 T cells activated by hepatocytes survived and became effective CTLs (Derkow et al. 2007; Klein and Crispe 2006; Wuensch et al. 2006). The reason for such opposing outcomes has not yet been elucidated but might be related to the use of TCR transgenic T cells with different affinities, antigen persistence, or even the frequency of antigen-expressing hepatocytes.

Although hepatocytes are not as accessible to circulating T cells as LSEC and KC, they might play an important role in presenting self-antigens to the immune system and inducing tolerance.

Primary T-Cell Activation in Liver, Table 1 Summary of the different immune molecules expressed by the different sinusoidal liver cells

Molecules	Hepatocytes	LSEC	KC	Liver cDCs	HSC
MHC I	+	+	+	+	+
MHCII	-	+	+	+	+
CD1d	+	+	+	+	+
CD80/CD86	-	+	+	+	+
CD40	-	+	?	+	+ (act)
B7H1	+	+	NA	+	+
TLRs (reported)	2, 3 and 4	1, 2, 3, 4, 6, 9	Most TLRs	most TLRs	2 (act), 4, 9

Please note that the list of TLRs is not exhaustive due to discrepancies in the literature.
Act activated stellate cells

In addition, it is likely that they would be the first cells to present viral antigens to T cells during infections by hepatotropic viruses (such as the hepatitis B and hepatitis C viruses). Thus, activation by hepatocytes might play an important role in shaping the initial antiviral immune response.

Stellate cells (*HSC*), also known as Ito cells, are located between LSEC and hepatocytes. Following liver damage and regeneration, they secrete the extracellular matrix that is critical for tissue repair. However, this process is also responsible for the development of fibrosis. In a non-inflamed liver, these cells play a role in regulating blood flow by constricting the diameter of the sinusoids (Rockey 2001). Cultured HSC express MHC class I and II, CD1, and low levels of CD80 and CD86 (Winau et al. 2007). Contact between HSC and T cells has not been directly observed; however, it is likely that they occur via LSEC fenestrations as demonstrated for hepatocytes. Although the role of these cells in intrahepatic T-cell activation has been largely ignored, recent studies have demonstrated that HSCs are also able to activate naïve CD8 T cells in situ (Winau et al. 2007).

Table 1 summarizes some of the immune molecules expressed by the different sinusoidal liver cells.

Physiological Role for Intrahepatic Primary T-Cell Activation

It is not clear why the liver has evolved the ability to activate the cellular adaptive immune response. The liver processes and excretes a wide variety of toxins and is exposed to pathogens and proteins circulating via the blood. An important function of the liver might thus be to trap blood-borne pathogens and to induce a local and rapid innate immune response that would eliminate the pathogen without requiring secondary lymphoid tissues. It is possible that KC play a role in this process.

The function of the liver in tolerance induction in transplantation and other settings is also puzzling and unresolved. This function might be related to the unusual location and drainage of this organ: in addition to the blood supply provided by the hepatic artery, the liver also receives venous blood from the gut and spleen carrying food antigens. This unique blood drainage has led investigators to speculate that the liver plays a critical role in oral tolerance. This hypothesis has been supported by experiments showing that an immune response against food was elicited when blood originating from the gut was surgically diverted to the systemic circulation, bypassing the liver. This possible role of the liver in oral tolerance remains to be verified.

Conclusion

The liver is an unusual immunological organ characterized by a unique blood flow and architecture that facilitates the interaction of liver resident cells with circulating leukocytes. Under low blood flow conditions, liver cells expressing p/MHC are able to retain and activate

naïve CD8 T cells in the absence of selectins. This property is unique among the solid organs. Although the outcome of such activation is controversial, it is likely that it will depend on the type of liver APC involved in the activation, the avidity of the interaction, and the presence or absence of inflammation.

Cross-References

- Acute and Chronic Hepatitis B Virus Infection, Immune Response
- Adaptive Immune Cells in the Liver
- Immune Responses to the Hepatitis C Virus
- Innate Immune Cells in the Liver
- Kupffer Cells in Immune Tolerance
- Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance
- Liver Transplantation Tolerance in Animal Models for Encyclopedia of Medical Immunology
- Liver Vasculature and Microvasculature

References

Ando K, Guidotti LG, Wirth S, Ishikawa T, Missale G, Moriyama T, et al. Class I-restricted cytotoxic T lymphocytes are directly cytopathic for their target cells in vivo. J Immunol. 1994;152:3245–53.

Beattie L, Peltan A, Maroof A, Kirby A, Brown N, Coles M, et al. Dynamic imaging of experimental Leishmania donovani-induced hepatic granulomas detects Kupffer cell-restricted antigen presentation to antigen-specific CD8 T cells. PLoS Pathog. 2010;6:e1000805.

Benseler V, Warren A, Vo M, Holz LE, Tay SS, Le Couteur DG, et al. Hepatocyte entry leads to degradation of autoreactive CD8 T cells. Proc Natl Acad Sci USA. 2011;108:16735–40.

Bertolino P, Bowen DG, McCaughan GW, Fazekas De St Groth B. Antigen-specific primary activation of CD8+ T cells within the liver. J Immunol. 2001;166:5430–8.

Bertolino P, McCaughan GW, Bowen DG. Role of primary intrahepatic T-cell activation in the 'liver tolerance effect'. Immunol Cell Biol. 2002;80:84–92.

Bertolino P, Schrage A, Bowen DG, Klugewitz K, Ghani S, Eulenburg K, et al. Early intrahepatic antigen-specific retention of naive CD8+ T cells is predominantly ICAM-1/LFA-1 dependent in mice. Hepatology. 2005;42:1063–71.

Bertolino P, Trescol-Biemont MC, Rabourdin-Combe C. Hepatocytes induce functional activation of naive CD8 + T lymphocytes but fail to promote survival. Eur J Immunol. 1998;28:221–36.

Bowen DG, Warren A, Davis T, Hoffmann MW, McCaughan GW, Fazekas De St Groth BF, et al. Cytokine-dependent bystander hepatitis due to intrahepatic murine CD8+ T-cell activation by bone marrow-derived cells. Gastroenterology. 2002;123:1252–64.

Bowen DG, Zen M, Holz L, Davis T, McCaughan GW, Bertolino P. The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. J Clin Invest. 2004;114:701–12.

Derkow K, Loddenkemper C, Mintern J, Kruse N, Klugewitz K, Berg T, et al. Differential priming of CD8 and CD4 T-cells in animal models of autoimmune hepatitis and cholangitis. Hepatology. 2007;46:1155–65.

Egen JG, Rothfuchs AG, Feng CG, Winter N, Sher A, Germain RN. Macrophage and T cell dynamics during the development and disintegration of mycobacterial granulomas. Immunity. 2008;28:271–84.

Holz LE, Benseler V, Bowen DG, Bouillet P, Strasser A, O'Reilly L, et al. Intrahepatic murine CD8 T-cell activation associates with a distinct phenotype leading to Bim-dependent death. Gastroenterology. 2008;135:989–97.

Holz LE, Benseler V, Vo M, McGuffog C, Van Rooijen N, McCaughan GW, et al. Naive CD8 T cell activation by liver bone marrow-derived cells leads to a "neglected" IL-2(low) Bim(high) phenotype, poor CTL function and cell death. J Hepatol. 2012;57(4):830–6.

Klein I, Crispe IN. Complete differentiation of CD8+ T cells activated locally within the transplanted liver. J Exp Med. 2006;203:437–47.

Klugewitz K, Adams DH, Emoto M, Eulenburg K, Hamann A. The composition of intrahepatic lymphocytes: shaped by selective recruitment? Trends Immunol. 2004;25:590–4.

Knolle PA, Schmitt E, Jin S, Germann T, Duchmann R, Hegenbarth S, et al. Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. Gastroenterology. 1999;116:1428–40.

Lee WY, Moriarty TJ, Wong CH, Zhou H, Strieter RM, van Rooijen N, et al. An intravascular immune response to Borrelia burgdorferi involves Kupffer cells and iNKT cells. Nat Immunol. 2010;11:295–302.

Limmer A, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, et al. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med. 2000;6:1348–54.

MacPhee PJ, Schmidt EE, Groom AC. Intermittence of blood flow in liver sinusoids, studied by high-resolution in vivo microscopy. Am J Physiol. 1995;269:G692–8.

MacSween RNM, Scothorne RJ. Developmental anatomy and normal structure. In: MacSween RNM, Anthony PP, Scheuer PJ, editors. Pathology of the liver. Churchill Livingstone: New York; 1979. p. 1–49.
Matsuno K, Ezaki T, Kudo S, Uehara Y. A life stage of particle-laden rat dendritic cells in vivo: their terminal division, active phagocytosis, and translocation from the liver to the draining lymph. [see comments.]. J Exp Med. 1996;183:1865–78.
McCuskey RS, Reilly FD. Hepatic microvasculature: dynamic structure and its regulation. Semin Liver Dis. 1993;13:1–12.
Mehal WZ, Juedes AE, Crispe IN. Selective retention of activated CD8+ T cells by the normal liver. J Immunol. 1999;163:3202–10.
Rockey DC. Hepatic blood flow regulation by stellate cells in normal and injured liver. Semin Liver Dis. 2001;21:337–49.
Warren A, Le Couteur D, Fraser R, Bowen DG, McCaughan GW, Bertolino P. T lymphocytes interact with hepatocytes through fenestrations in liver sinusoidal endothelial cells. Hepatology. 2006;44:1182–90.
Winau F, Hegasy G, Weiskirchen R, Weber S, Cassan C, Sieling PA, et al. Ito cells are liver-resident antigen-presenting cells for activating T cell responses. Immunity. 2007;26:117–29.
Wisse E, De Zanger RB, Charels K, Van Der Smissen P, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Hepatology. 1985;5:683–92.
Wong J, Johnston B, Lee SS, Bullard DC, Smith CW, Beaudet AL, et al. A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. J Clin Invest. 1997;99:2782–90.
Wuensch SA, Pierce RH, Crispe IN. Local intrahepatic CD8+ T cell activation by a non-self-antigen results in full functional differentiation. J Immunol. 2006;177:1689–97.

Prostaglandins, Leukotrienes, and Related Compounds

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Synonyms

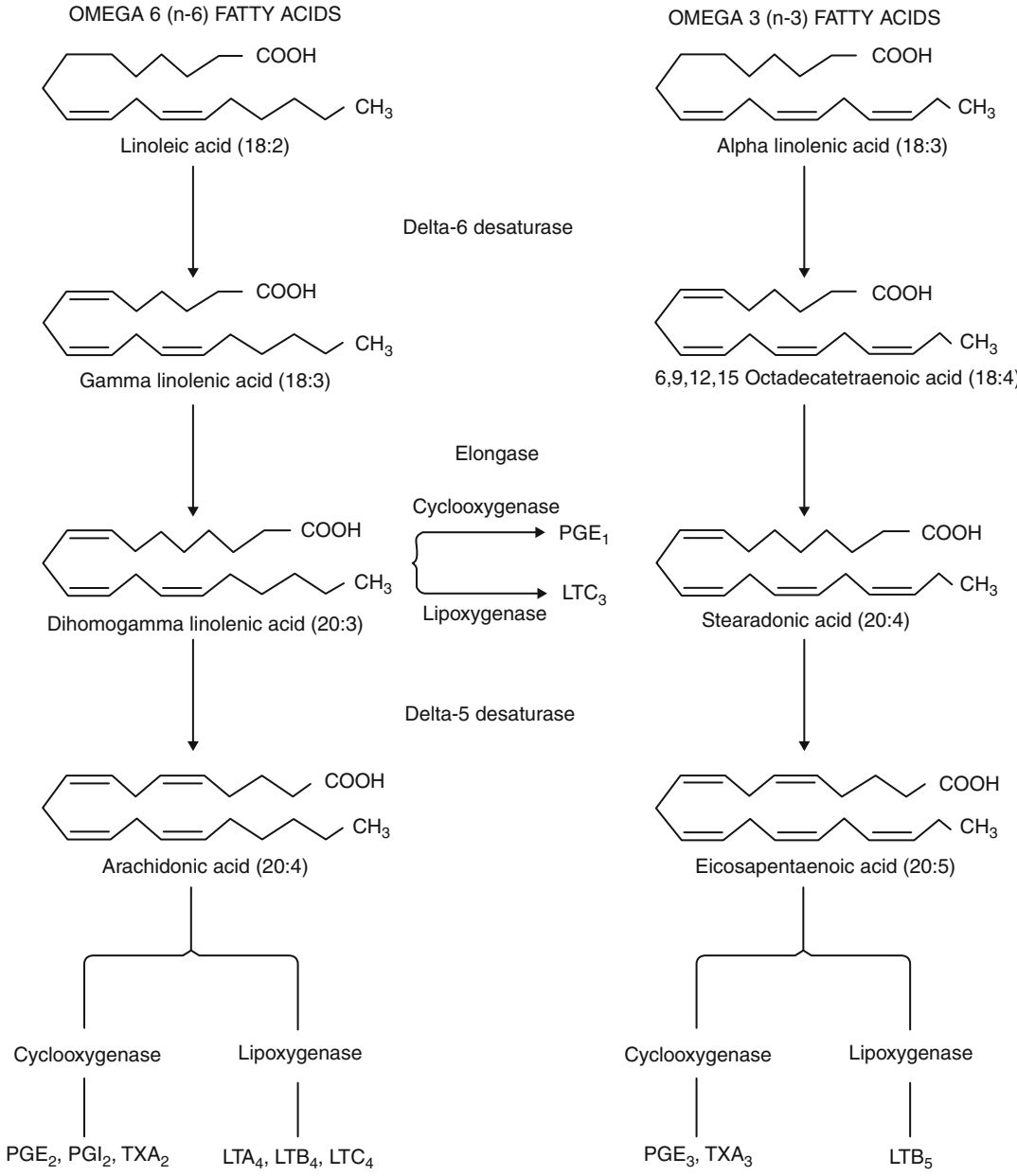
Eicosanoids

Definition

Over 80 years ago, it was observed that human myometrium exhibits rhythmic contraction and relaxation when incubated with fresh human seminal fluid. The active principle was then identified as an acidic lipid. Believing the lipid was produced in the prostate gland, von Euler named it *prostaglandin*. Eliasson later showed that seminal fluid prostaglandin in fact derives from the seminal vesicles. It was then determined that the active component in seminal fluid is not a single substance but several closely related compounds.

It is the oxygenation of several 20-carbon polyunsaturated fatty acids (arachidonic, dihomogammalinolenic, eicosapentaenoic) in nearly all human cell types that results in formation of several classes of bioactive products (Fig. 1) termed *eicosanoids* (eicosa = 20). These include prostaglandins (PGs), prostacyclin (PGI), thromboxanes (TX), leukotrienes (LT), and lipoxins (LX). All of these compounds are crucial to the regulation of immunity and inflammation, among other physiologic and pathologic processes.

Two groups of fatty acids are essential to the body: the omega-6 series derived from linoleic acid (18:2 n-6) and the omega-3 series derived from α-linolenic acid (18:3 n-3). The n refers to the number of carbon atoms from the methyl (omega) end of the fatty acid chain to the first double bond (i.e., omega-3 and omega-6 designations). Using this notation, 18 refers to the number of carbon atoms in the fatty acid. The degree of unsaturation (the number of double carbon-carbon bonds) follows the number of carbon atoms. Fatty acids are metabolized by an alternating sequence of desaturation (removal of two hydrogens) and elongation (addition of two carbons). Membrane phospholipids, the main storage site for polyunsaturated fatty acids, are particularly rich in eicosanoid precursors, which are located at the sn-2 position (Fig. 2). Because mammalian cells cannot interconvert n-3 and n-6 fatty acids, the composition of membrane phospholipids is determined by exogenous sources of fatty acids (Zurier 2009).

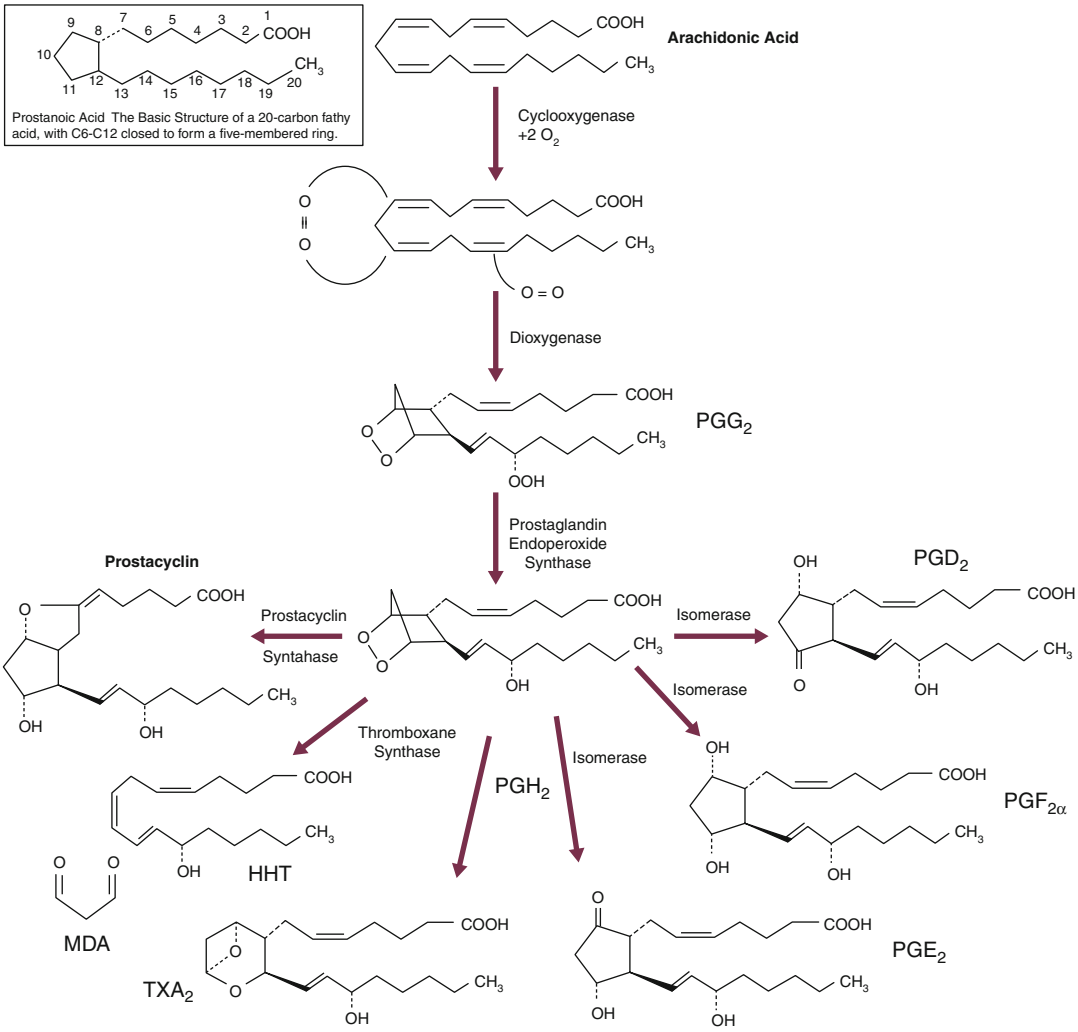


Prostaglandins, Leukotrienes, and Related Compounds, Fig. 1 Metabolic pathways of essential fatty acids. The pathways are ones of progressive desaturation alternating with elongation. Eicosanoid precursors include dihomogammalinolenic acid, arachidonic acid, and eicosapentaenoic acid. PG, prostaglandins; LT, leukotriene; TX, thromboxane

Biosynthesis of Eicosanoids

Phospholipases

Phospholipase A_2 (PLA_2) in lysosomes or bound to cell membranes catalyzes the breaking of the sn-2 bond, facilitating release of AA or other polyunsaturated fatty acids. The enzyme is crucial to regulation of eicosanoid synthesis because it is in the nonesterified state that the polyunsaturated precursors enter into the

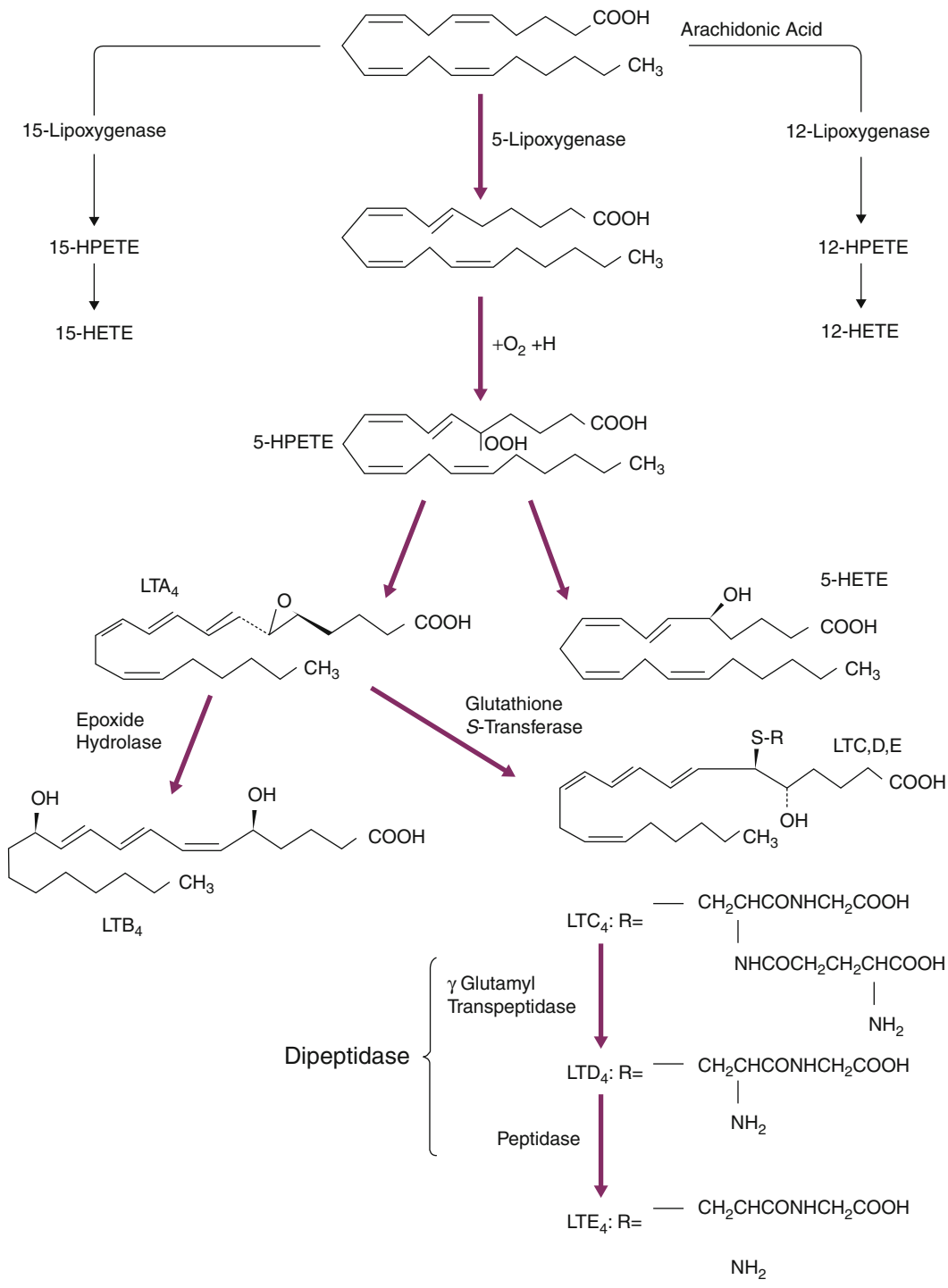


Prostaglandins, Leukotrienes, and Related Compounds, Fig. 2 Cyclooxygenase pathway of arachidonic acid metabolism. PG prostaglandin, MDA malondialdehyde, TX thromboxane

cascades leading to eicosanoid formation (Burke and Dennis 2009).

Under basal conditions, AA is liberated by $iPLA_2$, then reincorporated into cell membranes (reacylated), and is unavailable for appreciable eicosanoid biosynthesis. The acylase enzymes competitively inhibit the cyclooxygenase (COX) isoenzymes. After receptor activation and cell stimulation, intracellular Ca^{2+} levels increase, and the Ca^{2+}-dependent $cPLA_2$ liberates arachidonate at a rate that exceeds the rate of reacylation, leading to arachidonate metabolism by COX isoenzymes and by lipoxygenases.

The tetraenoic precursor (AA) is the most abundant of the three precursor fatty acids in cells of individuals who eat usual Western-style diets. Metabolites of AA constitute the "2" series (dienoic) PGs (two double bonds in the molecule), and the metabolic pathway has acquired the familiar name "arachidonic acid cascade." However, diets enriched in eicosapentaenoic or gamma-linolenic acid, the other eicosanoid precursors, lead to formation of different eicosanoids. The COX and 5-lipoxygenase pathways of the cascade are illustrated in Figs. 2 and 3, respectively.



Prostaglandins, Leukotrienes, and Related Compounds, Fig. 3 5-Lipoxygenase pathway of arachidonic acid metabolism. LT leukotriene, HETE hydroxyeicosatetraenoic acid, HPETE hydroperoxyeicosatetraenoic acid

Cyclooxygenase Pathway

The first step in the biosynthesis of the "prostanoids" (e.g., PGs, TXs, PGI) is catalyzed by the bifunctional PG endoperoxide synthase isozymes PGHS-1 (COX-1) and PGHS-2 (COX-2). To form the characteristic five-carbon ring structure (TXs contain a six-member ring), the precursor fatty acids must have double bonds at carbons 8, 11, and 14 (numbering from the carboxyl group). When a molecule of oxygen is inserted across carbons 9 and 11, ring closure occurs enzymatically across C8 and C12, creating the unstable PG endoperoxide PGG. Subsequent peroxidation yields PGH with formation of the cyclopentane ring. PGH serves as the common precursor for PGs, prostacyclin, and TXs that are formed under the influence of terminal synthases.

PGHS, the well-known target of nonsteroidal anti-inflammatory drugs (NSAIDs), exists in two isoforms. The localization of COX-1 in nearly all tissues under basal conditions suggests that its major function is to provide eicosanoids for physiologic regulation. This is seen clearly in platelets that do not have nuclei and cannot produce an inducible enzyme on activation. Rather, TXs are produced constitutively so that platelet aggregation can be completed (Rouzer and Marnett 2009).

The regulated formation of eicosanoids implies that cells have an appreciable ability to amplify the rate and amount of eicosanoid synthesis. Factors that regulate COX-2 expression are specific for the physiologic processes involved.

COX-1 and COX-2 effect a balance in several physiologic and pathologic situations. Of particular interest are their actions in kidney and stomach. During times of low blood volume, the kidney releases angiotensin and other factors to maintain blood pressure by systemic vasoconstriction. Angiotensin also provokes PG synthesis in the kidney. COX-1, expressed in vessels, glomeruli, and collecting ducts, produces vasodilating PGs, which maintain renal plasma flow and glomerular filtration during conditions of systemic vasoconstriction. In the antrum of the stomach, COX-1 leads to production of PGs, which increase gastric blood flow and mucus secretion. Inhibition of COX-1 by NSAIDs prevents these protective mechanisms and results in renal ischemia and damage and gastric ulcers (mainly antral) in susceptible individuals. These observations have led to development of NSAIDs that selectively inhibit COX-2 and spare COX-1. AA gains access to the active site of the COX via a hydrophobic channel, and access is blocked by insertion of an acetyl residue on Ser 530 in COX-1 and Ser 516 in COX-2. The irreversibility of the interaction and the unique expression of COX-1 in the anucleate platelet is the reason for the clinical efficacy of low-dose aspirin.

The major adverse effects of NSAIDs, gastroduodenal injury, impaired renal function, and induction of myocardial infarction and stroke are caused by inhibition of COX-1, whereas the analgesic and anti-inflammatory activities of NSAIDs rest in part on their ability to inhibit COX-2. COX-2 also seems to have a regulating role, however, in renal, brain, gastrointestinal, ovarian, and bone function. COX-2 also is expressed in endothelial cells, and its inhibition suppresses prostacyclin synthesis by endothelial cells. COX-2 acts in the initiation and the resolution of inflammation. Later in the response, COX-2 is expressed at even higher levels, leading to synthesis of PGD_2 and its dehydration product 15-deoxy-δ12,14-PGJ_2 (15δPGJ_2). Early expression of COX-2 is associated with production of inflammatory PGs, whereas the later peak results in production of PGs that suppress inflammation (Serhan 2011).

Acetaminophen, similar to NSAIDs, suppresses pain and fever. It is not an anti-inflammatory agent, and its mechanism of action – despite its extensive use – has not been apparent. The finding that acetaminophen inhibits COX activity more in canine brain homogenates than in spleen homogenates gave rise to the concept that variants of the COX enzyme (in addition to COX-1 and COX-2) exist that are differentially sensitive to acetaminophen. **COX-3** is a splice variant of COX-1.

COX-3 enzyme protein has been identified in human tissues, most abundantly in cerebral cortex and heart (Botting 2010).

Prostaglandin Synthases

Conversion of the endoperoxide intermediate PGH_2 to PGs requires the activity of specific terminal synthases: Hematopoietic PGD synthase (H-PGDS) catalyzes the isomerization of PGH_2 to PGD_2 in immune and inflammatory cells, cytosolic PGE synthase (cPGES) is responsible for constitutive expression of PGE_2, and microsomal PGE synthase (mPGES-1) induces PGE in response to inflammatory stimuli. Suppression of PG synthase activity might be considered as an alternative strategy that would fall between global blockade by inhibition of COX and blockade of a single eicosanoid receptor. Thus, efforts have been directed at development of drugs which inhibit mPGES-1, rather than COX-2, thereby suppressing PGE2 production while sparing production of prostacyclin (Claveau et al. 2003).

Products of the Cyclooxygenase Pathway

Prostaglandins

The basic structure of all PGs is a "prostanoic acid" skeleton, a 20-carbon fatty acid with a five-membered ring at C8 through C12 (Fig. 2, *inset*). The term *prostaglandins* is employed widely but should be used to describe only the oxygenation products that contain the five-membered carbon ring. The alphabetic PG nomenclature (e.g., PGE, PGF, PGD) is related to the chemical architecture of the cyclopentane ring. PGE and PGF differ only in the presence of a ketone or hydroxyl function at C9 (Fig. 2). These compounds are made by a variety of cells (e.g., PGE_2 and PGD_2 by isomerases, $PGF_{2\alpha}$ by a reductase). In the nomenclature, a subscript numeral after the letters indicates the degree of unsaturation in the alkyl and carboxylic acid side chains. The numeral *1* indicates the presence of a double bond at C13–C14 (PGE_1), *2* marks the presence of an additional double bond at C5–C6 (PGE_2), and *3* denotes a third double bond at C17–C18 (PGE_3).

PGs are produced on demand and seem to exert their effects on the cell of origin or nearby structures. Although lipophilic, the PGs do not leave the cell simply by diffusion. Rather, their release is mediated by the PG transporter, multidrug resistant protein 4 (MRP4), a member of the ATP-binding cassette transporter superfamily. They are not stored in cells and are degraded rapidly in vivo by 15-hydroxyprostaglandin dehydrogenase (PGDH) during one passage through the lungs. Abundant experimental evidence supports the view that PGs participate in development of the inflammatory response. PGs are probably better at potentiating the effects of other mediators of inflammation than they are at inducing inflammation directly. PGE compounds and intermediate hydroperoxides of AA increase pain sensitivity to bradykinin and histamine. The effects of PGE are cumulative, depending on concentration and time. Even very small amounts of PGs, if allowed to persist at the site of injury, may in time cause pain (Zurier 2009).

PGE_2 stimulates bone resorption (Blackwell et al. 2010), and its 13,14-dihydro derivative is nearly as potent, which is of interest because derivatives of the biologically active PGs are usually assumed not to be of functional significance. The observation that PGE_1 can stimulate bone formation suggests that PGs physiologically participate in coordination of bone formation and resorption.

PGD_2, the major PG formed by mast cells, also can mediate histamine release from mast cells exposed to anti-IgE antibody. PGJ_2, formed from the dehydration of PGD_2, seems to function as a brake on the inflammatory response. It reduces macrophage activation, reduces nitric oxide production from stimulated cells, and induces apoptosis in tumor cell lines. PGJ_2 is metabolized to 15-deoxy-δ12,14 PGJ_2 and δ12 PGJ_2, which also are biologically active (Zurier 2009).

Prostacyclin

Prostacyclin has been purified, and the cDNA for prostacyclin synthase has been cloned. In addition to a cyclopentane ring, a second ring

is formed by an oxygen bridge between carbons 6 and 9. It is generated from PGH_2 by a distinct prostacyclin synthase, a 56-kD member of the cytochrome P-450 superfamily of enzymes found predominantly in endothelial and vascular smooth muscle cells (Rouzer and Marnett 2009). Production of prostacyclin can be stimulated by thrombin or generated by transfer of PGH_2 from platelets (the endoperoxide steal), contact with activated leukocytes, or stretching of the arterial wall. It is a powerful vasodilator and inhibits platelet aggregation through activation of adenylate cyclase, which leads to an increase in intracellular cAMP. It is metabolized rapidly (half-life in plasma is less than one circulation time) to the more stable, less biologically active 6-keto-$PGF_{1\alpha}$. The enzymatic products of its conversion – 2,3-dinor-6 keto-$PGF_{1\alpha}$ and 6,15-di-keto- 2,3-dinor $PGF_{1\alpha}$ – also are chemically stable and have very little biologic activity. They are the major metabolites of prostacyclin excreted in urine, in which they can be assayed as indicators of prostacyclin generation.

Prostacyclin generated in the vessel wall has antiplatelet and vasodilator actions, whereas TXA_2 generated by platelets from the same precursors induces platelet aggregation and vasoconstriction. These two eicosanoids represent biologically opposite poles of a mechanism for regulating the interaction between platelets and the vessel wall and of formation of hemostatic plugs and intra-arterial thrombi. Given the central role of platelets in inflammatory reactions, an appropriate prostacyclin-TX balance is important to regulation of inflammation (Gryglewski and Mackiewicz 2010).

Thromboxanes

The endoperoxide PGH_2 can be converted into TXs after the action of the enzyme TX synthase, a microsomal 60-kD member of the cytochrome P-450 family, which is quite active in the platelet. The gene that encodes the enzyme has been cloned. TXs contain a six-member oxane ring instead of the cyclopentane ring of the PGs. TX synthase converts PGH_2 into equal amounts of TXA_2 and 12 L-hydroxy-5,8,10-heptadecatrienoic acid. TXA_2 stimulates platelet activation, contributes to intravascular aggregation of platelets, and contracts arteriolar and bronchiolar smooth muscles. It is hydrolyzed rapidly (half-life is 30 s) to the inactive, stable, measurable product, TXB_2; its actions are limited to the microenvironment of its release.

The extraordinary rapidity with which platelets adhere to damaged tissue, aggregate, and release potent biologically active materials suggests that the platelet is well suited to be a cellular trigger for the inflammatory process. Efforts directed at suppression of TX synthesis and platelet aggregation may result in limitation of inflammatory responses, especially in coronary arteries. Inhibition of platelet aggregation may be important to the anti-inflammatory effects of aspirin and other NSAIDs. Long-term administration of low doses of aspirin (40 mg/day – the lowest dose predicted to cause total inhibition of TX formation in serum, according to mathematic modeling) has inhibitory effects on platelet function ex vivo that are indistinguishable from the effects caused by giving 325 mg/day of aspirin. Aspirin acetylation of COX in platelets occurs in the portal vein where the aspirin concentration is high before it is metabolized in the liver, which explains why the 81 mg dose of aspirin is so effective in prevention of heart attacks and strokes. Platelets lose their ability to aggregate until new platelets are formed in about a week.

Selective inhibition of TX synthase represents an approach that can suppress TXA2 synthesis without depressing prostacyclin formation. Antagonists of the receptors shared by endoperoxide and TXA_2 have been developed, and these agents inhibit platelet aggregation in patients who are recalcitrant to TX synthase inhibition. Novel agents targeting TX receptors and TX synthase have improved treatment of vasculitis and cardiovascular and renal diseases (Zurier 2009; Rafferty and Walters 2010).

Lipoxygenase Pathways

In contrast to the COX pathway, in which stable products have three atoms of oxygen covalently attached to AA from 2 mol of molecular oxygen, lipoxygenases insert a single oxygen atom

into the molecular structure of AA. Separate lipoxygenases exist in certain cells and have strict structural requirements for their substrates. Six major mammalian lipoxygenases exist that insert their oxygen atoms into the 5, 12, or 15 position of AA, with formation of a new double bond and hydroperoxy group. Lipoxygenases that act on AA are found in the cytosol fraction of cells.

The human 5-lipoxygenase (5-LO) gene has been isolated and characterized and produces a 78-kD enzyme. In myeloid cells, the 5-lipoxygenase pathway leads to formation of the biologically active leukotrienes (LTs) (Fig. 3) that were originally found in leukocytes and that contain three conjugated double bonds (trienes). Cell activation leads to translocation of 5-lipoxygenase from cytosol to the nuclear membrane, where it encounters the 18-kD, 5-lipoxygenase-activating protein (FLAP). AA also is translocated to FLAP for presentation to 5-lipoxygenase (5-LO), In addition, upon cell stimulation, cPLA2 is activated and also associates with the nuclear membrane, close to FLAP. The ability of macrophages and dendritic cells to respond appropriately during innate immune responses is likely regulated by 5-LO and 12-LO.

The unstable HPETE is the initial metabolite of each lipoxygenase pathway. HPETE is reduced to the more stable HETE or is converted by 5-LO to LTA_4. LTA_4 can be converted to LTB_4 (in neutrophils and macrophages) or conjugated with reduced glutathione to form LTC_4 (in eosinophils, mast cells, endothelial cells, and macrophages). In contrast to lipoxygenase, which is mainly distributed in myeloid cells, LTA_4 hydrolase (5,12-dihydroxy-eicosatetraenoic acid), a zinc-requiring enzyme that converts LTA_4 to LTB_4, is widely distributed. From the cDNA sequence, it was suggested that mRNA for LTA_4 may have a short half-life, which could account for the properties of extremely rapid production and shutdown of LTB_4 and other eicosanoid biosynthesis.

LTA_4 can be exported from the cell of origin and converted in other cells by LTA_4 hydrolase to LTB_4. This transcellular metabolism also applies to conversion of LTA_4 to LTC_4 by LTC_4 synthase, a glutathione-*S*-transferase. LTD_4 and LTE_4 arise from LTC_4 after sequential removal of γ-glutamic acid and glycine from LTC_4. The enzyme γ-glutamyl transpeptidase is present in many cells as part of a complex enzymatic system involved in glutathione biosynthesis and amino acid transport. In many systems, the major sulfidopeptide LT has been reported to be LTD_4, rather than the precursor LTC_4. Removal of glycine from LTD_4 results in LTE_4 with concomitant loss of a significant amount of biologic activity (Radmark and Samuelsson 2010).

Products of the Lipoxygenase Pathways

The biologic effects of compounds produced in the lipoxygenase pathway indicate their importance in inflammatory diseases (Brash 1999). They are the major mediators of inflammation formed by the oxygenation of AA and are implicated as key mediators in several diseases, including inflammatory bowel disease, psoriasis, bronchial asthma, and RA.

5-HETE and 5-HPETE stimulate the generation of superoxide in human neutrophils. These compounds also augment intracellular calcium levels, facilitating PKC-dependent activation of a superoxide generating system of neutrophils. LTB_4 increases adherence of leukocytes to endothelial cells, a response that is augmented by exposure of the endothelial cells to TNF-α. LTB_4 does not seem to have a direct vascular contractile action because it is inactive in the hamster cheek pouch preparation and several other microvasculature systems. In rabbit skin, administration of LTB_4 with a vasodilator PG induces plasma exudation, which suggests that LTB_4 may facilitate enhanced vascular permeability. Increased venule permeability does occur in response to LTC_4, LTD_4, and LTE_4. LTB_4 is a potent chemotactic factor for neutrophils and is weakly chemotactic for eosinophils. LTB_4 and, to a lesser extent, 5-HETE enhance migration of T lymphocytes in vitro. LTB_4 also serves an immunoregulatory function. It stimulates differentiation of competent $CD8^+$ T lymphocytes from precursors lacking the CD8

marker. LTB_4 also stimulates interferon-γ and IL-2 production by T cells and biosynthesis of IL-1 by monocytes.

Strategies for inhibiting production or antagonizing the actions of LTs include development of selective LT receptor antagonists and inhibition of the production of LT by blocking the action of 5-lipoxygenase. Inhibition of enzymes distal in the LT cascade, such as LTA_4 hydrolase, also is a promising strategy for development of anti-inflammatory drugs. The existing agents may be aiming at the wrong target. Fibroblasts do not make much LTB_4, but they do make 12-HETE, which is a growth factor, through a cytochrome P-450 pathway. Cytochrome P-450 inhibitors may be more to the therapeutic point. Some inhibitors are more effective when activated by exposure to light. These inhibitors might prove useful as topical agents for treatment of inflammatory skin disease.

Lipoxygenase activities do not lead solely to production of mediators of inflammation. DGLA is converted by 15-lipoxygenase into 15-HETE, which is incorporated into diacylglyceride (DAG) and exerts anti-inflammatory effects partly by interfering with PKC-β activity. A lipoxygenase product of linoleic acid, 13-hydroxyoctadecadienoic acid, also suppresses inflammation and cell proliferation by means of a similar mechanism. EPA is converted by lipoxygenase into 15-hydroxyeicosapentaenoic acid, which also exhibits anti-inflammatory properties (Zurier 2009; Noguchi and Okubo 2011).

Lipoxins and Resolvins

(See ▶ Resolution of Inflammation)

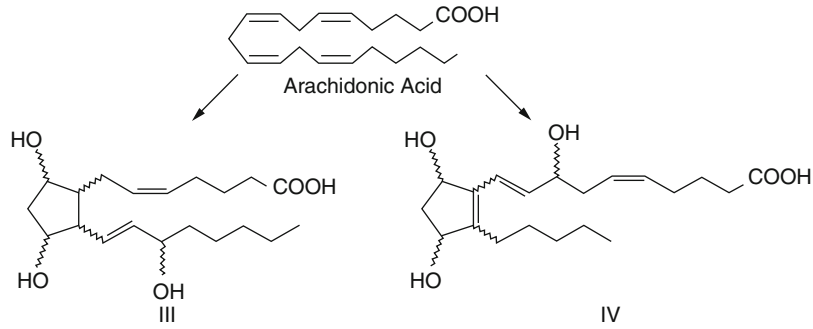
Isoeicosanoids

Isoeicosanoids, isomers of enzymatically derived eicosanoids, are derived from the free radical-mediated peroxidation of polyunsaturated fatty acids, including omega-3 fatty acids. They include members of the F, D, and E isoprostanes, isothromboxanes, and isoleukotrienes. Analysis of the isoprostanes (IsoP) indicates that they reflect lipid peroxidation in vivo. One F-type isoprostane, isoprostane F2α III (formerly 8-isoprostaglandin F2α) has been studied in detail because of its biologic activity in vitro. Isoprostane F2α III (Fig. 4) is a potent vasoconstrictor, with actions that are blocked by TX receptor antagonists. Although isoprostanes may act as ligands at TX or PG receptors (8,12-isoprostane F2α III activates the PGF2α receptor), they also may activate specific isoprostane receptors.

What all isoprostanes have in common, and what distinguishes them from the PGs, is the fact that the top (α) and the bottom (ω) side chains are always syn – that is, crowded together on the same face of the cyclopentane ring. In contrast to conventional, enzymatically derived PGs that are formed intracellularly and released immediately, isoprostanes are formed in the cell membrane where they remain until cleaved by phospholipases, circulate in plasma, and are excreted in urine. As stable end products of lipid peroxidation, endogenously formed IsoP are useful markers of oxidative stress and independent risk markers of coronary artery disease (Durand et al. 2010).

Endocannabinoids

Groups of naturally occurring members of the eicosanoid superfamily that can activate cannabinoid receptors and are derivatives of long-chain fatty acids have been referred to as endocannabinoids. They are not stored in cells. Rather, they are synthesized rapidly in the manner of PGs and LTs from lipid precursors and are released from – among other cells – cells of the immune system during immune/inflammatory responses. They can then activate cannabinoid receptors on the same or adjacent cells and are metabolized rapidly by the specific serine hydrolase, fatty acid amide hydrolase (FAAH), or by monoglyceride lipase or N-acylethanolamine. Thus, inhibition of FAAH offers a strategy for treatment of chronic inflammatory pain (Naidu et al. 2010). One of the most important endocannabinoids is anandamide (from the Sanskrit word for "bliss"), the amide conjugate of AA and ethanolamine (arachidonoyl ethanolamide).



Prostaglandins, Leukotrienes, and Related Compounds, Fig. 4 Isoprostane F2a structures. *Jagged lines* indicate that stereochemistry is uncertain

Anandamides and other endocannabinoids, such as 2-arachidonoylglycerol and virodhamine, are involved in a wide range of regulatory functions including pain perception and modulation of immune responses, actions mediated via cannabinoid 1 (CB1) and CB2 receptor subtypes, resulting in activation of G proteins of the G(i/o) family.

Acid congeners of anandamides are lipoamino acids (elmiric acids) that exist as endogenous substances, regulate tissue levels of anandamide, and exhibit anti-inflammatory effects and the capacity to facilitate resolution of inflammation. One such compound, N-arachidonoyl glycine (NAGly), is found in many tissues at higher concentrations than anandamide. It has analgesic actions similar to those reported for anandamide but does not exhibit psychotropic action. A library of elmiric acids (Burstein 1999) (n-3 and n-6) has been synthesized. Several of these compounds exhibit anti-inflammatory activity, likely in part by increasing production of PGJ2. That anandamide can enhance its own synthesis in macrophages suggests the presence of a rapid response to counter excessive inflammatory or immune responses. Anandamide is converted by COX-2 (but not by COX-1) into PGE_2 or $PGF_{2\alpha}$ ethanolamide directly, without going through free AA. These novel PGs ("prostamides") are pharmacologically active. Because anandamide is a substrate for COX-2, inhibitors of COX-2 may reduce anandamide metabolism with a subsequent increase in concentration of the anandamide. A combination of anandamide with ibuprofen produces synergistic analgesia in rats (Guindon et al. 2006).

Eicosanoid Receptors

For many years, it was thought that the lipophilic eicosanoids – in contrast to the peptide molecules for which receptors were characterized routinely – simply "diffused" into cell membranes or were carried in by a binding protein. The isolation and cloning of eicosanoid receptors changed that thinking.

Prostaglandin Receptors exert most of their actions through G protein-coupled receptors. Receptors for the COX products are designated P receptors, depending on the prostanoid that has most affinity for them. These receptors include the PGD receptors (DP), four subtypes of the PGE receptor (EP1 through EP4), the PGF receptor (FP), the PGI2 receptor (IP), and the TX receptor (TP). The IP, DP, EP2, and EP4 receptors mediate increases in cellular cyclic AMP, whereas TP, FP, and EP1 receptors induce calcium mobilization. EP2, EP4, and IP regulate macrophage cytokine production in a similar manner. As might be expected, signaling through these receptors is more complicated (Ricciotti and Fitzgerald 2011; Shimizu 2009).

Leukotriene Receptors

Surface receptors for LTB_4 denoted BLT1 and BLT2 and for the cysteinyl LTs also exert their actions through transmembrane-spanning G protein-coupled protein receptors. High-affinity LTB_4 receptors transduce chemotaxis and adhesion

responses, whereas low-affinity receptors are responsible for secretion of granule contents and superoxide generation. A COX-1 derived ligand 12(S)-hydroxyheptadeca-5Z,8E,10E-trienoic acid (12-HHT), produced during thromboxane synthesis, is an endogenous high-affinity ligand for BLT2 (now called BLT2/HHTR), another example of a connection between the LO and COX pathways. The cysteinyl LTs are recognized by at least two GPCR denoted CysLT1 and CysLT2. CysTL1 mediates calcium mobilization and inhibition of adenylate cyclase, whereas CysTL2 mediates calcium mobilization and increased cyclic AMP concentrations. The preferred ligands for CysLT1 are LTD4 > LTC4 > LTE4. CysLT2 binds LTC4 and LTD4 equally, whereas LTE4 exhibits low affinity for the receptor. Both receptors have wide tissue and cellular distribution, including a presence in cells which participate in immune responses. More than a dozen chemically distinct, specific, and selective antagonist drugs that block the binding of LT to $CysLT_1$ have been identified. Clinical use of these compounds has mainly been in the treatment of asthma (Mathis et al. 2010).

Lipoxin Receptors

Lipoxins can act at their own specific receptors for LXA_4 and LXB_4, and LXA_4 can interact with a subtype of LTD_4 receptors. Lipoxins also can act at intracellular targets within their cell of origin or after uptake by another cell. The monocyte and neutrophil LXA_4 receptors are identical at the cDNA level, but they evoke different responses, and the LXA_4 receptor on endothelial cells seems to be a structurally distinct form. LXA4 also binds to the human orphan receptor GPR32, a member of the chemoattractant receptor family (Chiang et al. 2006).

Nuclear Receptors

Nuclear receptors are a superfamily of ligand-regulated transcription factors that interact with other transcription factors and with co-regulators that either enhance (co-activators) or inhibit (corepressors) transcription. The major nuclear receptors involved in regulation of inflammation are the glucocorticoid receptor (GR), peroxisome proliferator-activated receptors, liver X receptors (LXR), and the orphan receptor nuclear receptor-related 1 protein (Nurr1). Other members of the nuclear receptor family which contribute to the regulation of inflammation include estrogen receptors, vitamin D receptor, and retinoic acid receptors (Huang and Glass 2010).

Platelet-Activating Factor

Platelet-activating factor (PAF, 1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine), is a potent mediator of inflammation that causes neutrophil activation, increased vascular permeability, vasodilation, and bronchoconstriction in addition to platelet activation. PAF is formed by a smaller number of cell types than the eicosanoids: mainly, leukocytes, platelets, and endothelial cells. Because of the extensive distribution of these cells, however, the actions of PAF can manifest in virtually every organ system. In contrast to the two long-chain acyl groups present in phosphatidylcholine, PAF contains a long-chain alkyl group joined to the glycerol backbone in an ether linkage at position 1 and an acetyl group at position 2. PAF represents a family of phospholipids (PAF-like lipids: PAF-LL) because the alkyl group at position 1 can vary in length from 12 to 18 carbons. PAF, similar to the eicosanoids, is not stored in cells. Rather, it is synthesized when cells are stimulated, at which time the composition of the alkyl group may change. The immediate effects of PAF are mediated through a cell surface G protein-coupled receptor PAFR. PAFR is coupled to Gi, Gq, and G12/13. Activation of PAFR results in inhibition of cyclic AMP, mobilization of calcium, and activation of mitogen-activated protein kinases.

The synthesis of PAF is tightly regulated, and a family of intra- and extracellular phospholipases A2 (PAF acetylhydrolases; PAF AH) degrades PAF and PAF-LL, thereby regulating their half-life and their engagement with the PAFR. In addition, receptor desensitization controls binding of PAF to its receptor (Yost et al. 2010).

Cross-References

- CD40
- CD5
- Cell Adhesion Molecules
- Chemokines
- CTLA-4
- Fibrosis
- Neutrophils
- Resolution of Inflammation
- Systemic Lupus Erythematosus, Pathogenesis
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- Tregs in the Liver

References

Blackwell CA, Raisz LG, Pilbeam CC. Prostaglandins in bone: bad cop, good cop? Trends Endocrinol Metab. 2010;21:294–301.

Botting RM. Vane's discovery of the mechanism of action of aspirin changed our understanding of its clinical pharmacology. Pharmacol Reports. 2010;62:518–25.

Brash AR. Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem. 1999;274:23679–82.

Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res. 2009;50:S237–42.

Burstein S. The cannabinoid acids: non psychoactive derivatives with therapeutic potential. Pharmacol Therap. 1999;82:87.

Chiang N, Serhan CN, Dahlen S-E, et al. The lipoxin receptor ALX: potent ligand specific and stereoselective actions in vivo. Pharmacol Rev. 2006;58:463.

Claveau D, Sirinyan M, Guay J, et al. Microsomal prostaglandin E synthase-1 is a major terminal synthase that is selectively up-regulated during cyclooxygenase-2-dependent prostaglandin E2 production in the rat adjuvant-induced arthritis model. J Immunol. 2003;170:4738–44.

Durand T, Bultel-Ponce V, Guy A, et al. Isoprostanes and phytoprostanes: bioactive lipids. Biochimie. 2010;93:52–60.

Gryglewski RJ, Mackiewicz Z. Vane's blood bathed organ technique adapted to examine the endothelial effects of cardiovascular drugs in vivo. Pharmacol Rep. 2010;62:462–7.

Guindon J, DeLean A, Beaulieu P. Local interactions between anandamide, an endocannabinoid, and ibuprofen, a nonsteroidal anti-inflammatory drug, in acute and inflammatory pain. Pain. 2006;21:85–93.

Haeggstrom JZ, Rinaldo-Mathis A, Wheelock C, et al. Advances in eicosanoid research, novel therapeutic implications. Biochem Biophys Res Commun. 2010;396:135–9.

Huang W, Glass CK. Nuclear receptors and inflammation control: molecular mechanisms and pathophysiological relevance. Arterioscler Thromb Vasc Biol. 2010;30:1542–9.

Mathis SP, Jala VR, Lee D, et al. Nonredundant roles for leukotriene receptors BLT1 and BLT2 in inflammatory arthritis. J Immunol. 2010;185:3049–56.

Naidu PS, Kinsey SG, Guo TL, et al. Regulation of inflammatory pain by inhibition of fatty acid amide hydrolase. J Pharmacol Exp Ther. 2010;334:182–90.

Noguchi K, Okubo M. Leukotrienes in nociceptive pathway and neuropathic/inflammatory pain. Biol Pharm Bull. 2011;34:1163–9.

Radmark O, Samuelsson B. Regulation of the activity of 5-lipoxygenase, a key enzyme in leukotriene biosynthesis. Biochem Biophys Res Commun. 2010;396:105–10.

Rafferty M, Walters MR. Anti-platelet therapy and aspirin resistance- clinically and chemically relevant? Curr Med Chem. 2010;17:4578–86.

Ricciotti E, Fitzgerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011;31:986–1000.

Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. J Lipid Res. 2009;50:S29–34.

Serhan CN. The resolution of inflammation: the devil in the flask and in the details. FASEB J. 2011;25:1441–8.

Shimizu T. Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. Annu Rev Pharmacol Toxicol. 2009;49:123–50.

Yost CC, Weyrich AS, Zimmerman GA. The platelet activating factor (PAF) signaling cascade in systemic inflammatory responses. Biochimie. 2010;92:692–7.

Zurier RB. Prostaglandins, leukotrienes, and related compounds. In: Firestein GS, Budd RC, Harris Jr ED, McInnes IB, Ruddy S, Sergent JS, editors. Kelley's textbook of rheumatology. 8th ed. Philadelphia: Saunders Elsevier; 2009. p. 343–56.

Proteinuric Kidney Diseases: Importance of Blood Pressure Control

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Synonyms

Blood pressure; Chronic kidney disease; Glomerulonephritis; Hypertension; Proteinuria

Definitions

Hypertension is defined as blood pressure elevation to a level that places patients at risk for target organ damage including the kidneys, heart, and brain. The Joint National Committee on Hypertension defines hypertension for the general population as systolic blood pressure (SBP) greater than 140 mm Hg or diastolic blood pressure (DBP) greater than 90 mm Hg (Chobanian et al. 2003). Chronic kidney disease (CKD) is defined by the Kidney Disease Quality Outcome Initiative (K/DOQI) as either kidney damage or a glomerular filtration rate (GFR) of less than 60 ml/min/1.73 m^2 for 3 months or more irrespective of cause. Kidney damage refers to demonstrated pathologic abnormalities or the presence of makers of damage, including a urine albumin-to-creatinine ratio greater than 30 mg/g (Levey et al. 2004). In CKD, hypertension is considered to be a blood pressure greater than 130/80 mm Hg (Levey et al. 2004). In addition to a more stringent level of blood pressure control in CKD, the choice of antihypertensive medications is important as some classes not only reduce blood pressure but are also kidney protective. These themes are developed in this entry. To the best of our knowledge, there are no prospective randomized trials on the effect of blood pressure control in immune-mediated kidney diseases. Thus, our recommendations are based on the randomized trials for blood pressure control in other forms of CKD. Our experience in managing blood pressure in immunologic kidney disorders supports the notion that this is a valid extrapolation.

Introduction

Hypertension is a major modifiable risk factor for the development and progression of CKD. It is present in 85 % of patients with CKD and the prevalence of hypertension increases as glomerular filtration rate falls. Progressive kidney disease makes blood pressure more difficult to control. Also, hypertension superimposed on kidney disease promotes renal arteriolar nephrosclerosis and glomerular damage. This leads to a vicious cycle as kidney disease worsens hypertension and hypertension accelerates kidney disease (Sarafidis et al. 2007). Further, hypertension worsens proteinuria because it raises intraglomerular pressure. Proteinuria is the strongest single risk factor for CKD progression (progressive GFR decline). So, blood pressure control is essential to control proteinuria and slow CKD progression. Hypertension in immunologic kidney disorders such as glomerular disease is usually triggered by two factors: volume expansion and renal ischemia leading to activation of the renin-angiotensin-aldosterone system (RAAS). Unchecked upregulation of the RAAS leads to activation of several pathways deleterious to kidney function, including profibrotic growth factors, proinflammatory cytokines, and prothrombotic molecules.

CKD Progression

"Natural progression" of CKD refers to the decline in kidney function that is attributable to

mechanisms independent from those of the patient's original kidney disease. CKD patients are vulnerable to natural progression after they have sustained a GFR loss of greater than 50 % (Brown et al. 2010). The mechanisms of natural progression are largely related to overperfusion of the surviving glomeruli, which raises intraglomerular pressure and increases proteinuria. This mechanism, working in concert with metabolic derangements found in CKD such as hyperlipidemia, hyperuricosuria, and hyperphosphatemia, facilitates natural progression.

Another mechanism of GFR decline is that attributable to aging. This occurs in those with normal kidney function and causes GFR loss of about 1 ml/min/year starting after the age of 45 (Brown et al. 2010). In patients with CKD, it is likely that natural progression is additive to the progression due to aging.

Whether progression due to aging can be slowed is not clear. However, there is abundant evidence that natural progression can be slowed. Generally, natural progression is slow, so even small improvements in slowing natural progression can result in large delays in time to end-stage renal disease (ESRD) and need for dialysis.

Blood pressure control and reduction of proteinuria are critical to delay CKD progression. Four major trials provided blood pressure targets in CKD patients stratified by level of proteinuria (Klahr et al. 1994) (Wuhl et al. 2009; Wright 2002; Ruggenenti et al. 2005). Patients with proteinuric CKD (>1 g/day proteinuria) should have blood pressure controlled to less than 125/75 mm Hg. In addition, in patients with proteinuric kidney disease, the worse the proteinuria, the greater the benefit from aggressive blood pressure reduction on CKD progression. Those with less than 1 g/day proteinuria should continue to have blood pressure controlled to less than 130/80 mm Hg.

CKD Progression and the Renin-Angiotensin-Aldosterone System (RAAS)

Inhibition of the RAAS is of particular importance to delaying CKD progression. Protein excretion varies directly with intraglomerular pressure, and blockade of the RAAS decreases efferent arteriolar tone, thus decreasing glomerular capillary hydrostatic pressure (Yoshioka et al. 1987). The RAAS may also cause proteinuria by direct effects on podocytes (Durvasula et al. 2004). Intrarenal activation of the RAAS mediates podocyte apoptosis via angiotensin receptor 1 (ATR1) and may affect nephrin expression, disrupting slit diaphragm integrity on podocytes causing a loss in the ability to restrict protein filtration (Benigni et al. 2001; Bonnet et al. 2001). Furthermore, angiotensin II promotes glomerulosclerosis by promoting glomerular cell proliferation, altering growth factor expression and activating proinflammatory cytokines (Fogo 2006; Ruiz-Ortega et al. 2006). RAAS inhibition mitigates these maladaptive changes and helps restore podocyte health by reestablishing vascular endothelial growth factor (VEGF-A) and angiopoeitin-1 activity and inhibiting profibrotic factors, such as plasminogen activator inhibitor 1 (PAI-1) and transforming growth factor beta (TGF-β) (Table 1) (Brewster and Perazella 2004).

Angiotensin-Converting Enzyme Inhibitor (ACEi) Therapy

ACEi suppress the RAAS thus reducing blood pressure and proteinuria and restoring glomerular structure and function. Several large trials have validated the benefit of ACEi in proteinuric kidney disease (Lewis et al. 1993; The GISEN 1997; Maschio et al. 1996). Each trial demonstrated a significant reduction in proteinuria, rate of CKD progression, and onset of ESRD in patients treated with ACEi compared to placebo. A placebo-controlled trial of nondiabetics with CKD demonstrated a 53 % reduction in doubling of serum creatinine or need for dialysis after three years of follow-up in those receiving benazepril. The effect was greatest in those with proteinuria >1 g/day (Maschio et al. 1996).

In patients with proteinuric kidney disease, ACEi should be considered first-line therapy for hypertension and are also recommended in normotensive, proteinuric patients (Table 2). In both cases the ACEi should be titrated to the

Proteinuric Kidney Diseases: Importance of Blood Pressure Control, Table 1 Negative effect of the renin-angiotensin-aldosterone system (RAAS) on the kidney and heart

Dysregulated growth factors and cytokines[a]		Growth factor and cytokine effect	Result
Upregulated	*Downregulated*	1. Increased oxidative stress	*Renal*
TGF-B	Nitric oxide	2. Podocyte apoptosis	Glomerulosclerosis
CTGF	VEGF	3. Increased inflammation	Interstitial fibrosis
PAI-1	Angiopoeitin-1	4. Renal arteriolar vasoconstriction	*Cardiac*
MCP-1			Left ventricular hypertrophy
CCL5			Cardiac fibrosis
IL-8			
IL-6			
TNF-alpha			

[a]*TGF-B* Transforming growth factor beta, *CTGF* Connective tissue growth factor, *PAI-1* Plasminogen activator inhibitor, *MCP-1* Monocyte chemoattractant protein, *CCL5* C-C motif ligand 5, *TNF-alpha* Tumor necrosis factor alpha, *VEGF* Vascular endothelial growth factor

maximum tolerated dose, as there is a dose-dependent increase in anti-proteinuric effect, and this will limit risk of aldosterone escape (increasing aldosterone levels during ACEi therapy), which can attenuate ACEi effect (Struthers 1995; MacFadyen et al. 1999; Hou et al. 2007). After initiation of ACEi therapy, an increase in serum creatinine may occur due to reduced filtration pressure from decreased efferent arteriolar tone. Patients must be monitored closely for progressive rise in serum creatinine and side effects such as hyperkalemia (Bakris and Weir 2000). ACEi should be held if acute kidney injury develops for any reason and 48 h before major surgery or iodinated contrast procedures.

Angiotensin Receptor Blocker (ARB)

While the action of ARBs appears to be similar to ACEi, there are some important differences. ARBs act on the angiotensin 1a receptor (ATR1a) in the kidney, inhibiting the effects of angiotensin II and thus do not increase bradykinin and have less effect on aldosterone (Tsouli et al. 2006). Two large, placebo-controlled randomized clinical trials confirmed the renoprotective benefits of ARBs in patients with type II diabetic nephropathy (Brenner et al. 2001; Lewis et al. 2001). ARBs were found to have similar antihypertensive and anti-proteinuric effects as ACEi in patients with diabetic nephropathy.

There are no large randomized trials of ARBs in nondiabetic kidney disease. However, given the best available evidence, ARBs may be used as an alternative to individuals intolerant of ACEi. As with ACEi, patients should be monitored closely for side effects such as hyperkalemia and progressive increase in serum creatinine while on ARB therapy.

Combination ACEi plus ARB Therapy

The addition of an ARB to ACEi may be more anti-proteinuric than either agent alone. Several small trials have found a significant reduction in proteinuria and rate of GFR decline compared to monotherapy MacKinnon et al. (2006); Kincaid-Smith et al. 2002; Luno et al. 2002; Wolf and Ritz 2005). However, a recent randomized, placebo-controlled trial which evaluated 25,620 individuals with vascular disease or diabetes found an *increased* risk of reaching all renal end points with combination therapy despite achieving a lower blood pressure than with either ACEi or ARB alone. There were also more adverse events with combination therapy (Messerli et al. 2008b). While this is contrary to the previous smaller studies, this report should not be taken lightly. This patient population likely had significant underlying vascular disease and, by extension, presumed renovascular disease. If so, it is understandable that lower blood pressures could have resulted in worse renal outcomes. Additionally, the trial did not demonstrate additional cardiovascular benefit with combination therapy. Another large trial also found no additional

Proteinuric Kidney Diseases: Importance of Blood Pressure Control, Table 2 Antihypertensive therapies and prevention of CKD progression

Intervention	Goals/comments
ACEi therapy	First-line therapy for hypertension in proteinuric kidney disease
ARB therapy	First choice for ACEi intolerance
Combination ACEi + ARB	May consider in patients with proteinuria > 3 g/day that cannot be controlled by a single agent. Avoid in patients with renovascular or cardiovascular disease
Aldosterone blockade	Anti-proteinuric effect at low doses. Slows progression of CKD. Consider as second-line agent for proteinuric kidney disease. Avoid if GFR < 30 ml/min/1.73 m^2
NDCAs	Anti-proteinuric effect. May be used in addition to ACEi/ARB to further reduce proteinuria and control blood pressure
Avoid DCAs	Not anti-proteinuric, may worsen proteinuria and promote kidney disease progression. Use only for refractory hypertension
Restrict NaCl intake	Goal 80–120 mmol/day (2–3 g/day) helps with blood pressure control and will optimize anti-proteinuric and antihypertensive therapies
Diuretics	Recommended use in conjunction with RAAS inhibition for salt-sensitive hypertension and in those with refractory high-salt diet or nephrotic syndrome
B-blockers	Concern for diabetic risk. Recommended in patients with cardiovascular disease or 4th-line agent for blood pressure control

cardiovascular benefit with combination therapy immediately post-myocardial infarction and instead showed increased adverse events (Pfeffer et al. 2003).

Combination therapy with ACEi and ARB must be used with caution. Combination therapy may still be warranted in those with heavy proteinuria (>3gm/day) after either drug alone has been maximized. Patients must be closely monitored for side effects and this regimen should generally be avoided in patients with atherosclerotic renal disease and cardiovascular disease.

Renin Inhibitors

Aliskiren is an agent that directly inhibits renin activity and is a therapy that is being used more commonly in clinical practice. Two large studies recently evaluated the efficacy of direct renin inhibition combined with ACEi or ARB (Parving et al. 2008; Peterson 2011). The results were conflicting and one study was stopped early due to increased renal and cardiovascular adverse events with combination therapy (Peterson 2011). Overall, the role of aliskiren in nondiabetic kidney disease is untested and it should not be used in place of ACEi/ARBs or in combination with ACEi/ARB.

Aldosterone Antagonism

Aldosterone plays an important role in the pathogenesis of renal injury. In addition to the adrenal glands, aldosterone is synthesized in endothelial and vascular smooth muscle cells (Hollenberg 2004). It functions in the kidney to maintain salt and water homeostasis and assist with excretion of potassium.

Aldosterone promotes fibroblast and/or myofibroblast growth and, through TGF-β, regulates collagen deposition in blood vessels and the heart (Brilla 2000) (Sun et al. 2000). Within the kidney, aldosterone mediates vascular remodeling, and excess aldosterone expression promotes tubulointerstitial fibrosis in patients with kidney damage (Sun et al. 2000). Aldosterone antagonists, even in low doses, have anti-proteinuric, antihypertensive, antifibrotic, and cardioprotective effects (Zannad et al. 2011; Pitt et al. 1999; Chrysostomou and Becker 2001; Sato et al. 2003). ACEi provide some blockade of aldosterone action and work synergistically with aldosterone antagonists (Chrysostomou and Becker 2001). Directly antagonizing aldosterone may therefore be important adjunctive therapy to primary RAAS inhibition in patients with kidney disease.

While there are no strong clinical trial data with hard end points like ESRD, the available data support the use of aldosterone antagonists in patients with CKD who have not met blood pressure or proteinuria goals with ACEi/ARBs. Patients with CKD on aldosterone antagonists must be monitored closely for hyperkalemia,

especially if on concomitant ACEI/ARB therapy. Further, aldosterone antagonists should not be used in patients with GFR < 30 ml/min/1.73 m^2 due to risk for hyperkalemia.

Diuretics and Salt

Sodium Restriction

Increased salt intake worsens hypertension and proteinuria in patients with CKD (Jones-Burton et al. 2006). A high-salt diet can also override the effects of anti-proteinuric and antihypertensive therapy (Heeg et al. 1989; Weir et al. 1998; Bakris and Smith 1996). The effect of lisinopril on reduction in proteinuria was found to be strongly dependent on dietary sodium intake in one study (Heeg et al. 1989). Similarly, the addition of salt restriction and diuretics to ARB therapy reduced proteinuria 25 % more than the ARB alone, and patients who did not initially respond to losartan monotherapy for proteinuria did so after the addition of salt restriction (Vogt et al. 2008). The average adult American diet contains about 170 mmol/d or 3.9 g/d of sodium. In individuals with glomerular disease and proteinuria/hypertension, sodium intake should be restricted to 80–120 mmol/day (2–3 g/day). Salt intake can be monitored by measuring sodium in a 24-h urine collection.

Diuretics

Diuretic therapy synergizes with RAAS inhibition to improve blood pressure and reduce proteinuria (Vogt et al. 2008). Diuretics alone have not been shown to reduce proteinuria beyond what is achieved by blood pressure control. Diuretics such as the thiazides have significant metabolic side effects such as hypokalemia, hyperglycemia, hyperuricemia, hyperlipidemia, hyponatremia, and stimulation of the RAAS. Most of these side effects are known to increase cardiovascular risk. However, if diuretics are used in conjunction with an ACEi or ARB, these metabolic effects are mitigated (Messerli et al. 2008a). For patients with nephrotic syndrome, who are prone to volume retention, diuretic therapy is beneficial, especially in combination with RAAS blockade (Messerli et al. 2008a). If the GFR is less than 30 ml/min/1.73 m^2, then a loop diuretic (e.g., furosemide) is preferred over thiazides. Electrolytes should be monitored closely.

Calcium Channel Blockers

Non-Dihydropyridine Calcium Channel Antagonists (NDCA)

The NDCAs, including verapamil and diltiazem, have been found to be anti-proteinuric. They have also been found to slow decline in kidney function (Bakris et al. 1996, 2004). In a study of patients with overt diabetic nephropathy, NDCAs had anti-proteinuric and antiprogression effects similar to lisinopril and superior to atenolol (Bakris et al. 1997). Individuals who remain hypertensive with persistent proteinuria despite adequate RAAS inhibition may benefit from the addition of NDCAs, particularly if the GFR < 30 ml/min.

Dihydropyridine Calcium Channel Antagonists (DCA)

As opposed to NDCAs, DCAs, while extremely effective antihypertensive agents, decrease glomerular afferent arteriolar resistance and thus tend to increase glomerular capillary hydrostatic pressure. This can worsen proteinuria and accelerate loss of renal function (Bakris et al. 2004; Ruggenenti et al. 1998; Gashti and Bakris 2004; Hummel et al. 2010). A more recent study compared candesartan to amlodipine in hypertensive patients with moderate to advanced CKD. After a 3.2 year median follow-up, the candesartan group had slower progression of CKD, less new onset diabetes, and significantly less cardiovascular risk compared to the amlodipine group (Saruta et al. 2009). In patients with proteinuric kidney disease, these agents should be reserved for those with refractory hypertension.

Other Antihypertensives

Beta-Blockers

There is no evidence to suggest that β-blockers slow progression of CKD. β-blockers are

typically reserved for patients with underlying cardiac disease and are otherwise considered fourth-line therapy for blood pressure control. β-blockers may increase the risk for developing diabetes when used as monotherapy (Messerli et al. 2008a). β-blocker use is recommended for patients with underlying cardiac disease and may be considered in those with refractory hypertension.

Conclusions

Hypertension is common in immunologic kidney disease and is typically caused by prolonged tissue ischemia leading to RAAS activation and volume expansion. The objective of blood pressure control is to delay kidney disease progression and reduce cardiovascular risk. Proteinuria and blood pressure are linked as risk factors for CKD progression, and emphasis should be placed on treating hypertension with agents that are known to reduce proteinuria and slow progression of CKD. Inhibition of the RAAS is necessary to achieve these goals. ACEi are considered first-line therapy for hypertension in patients with proteinuric kidney disease with goal blood pressure less than 125/75 mm Hg, as tolerated. ARBs may be used if ACEi are not tolerated. Additional antihypertensive agents are often needed and those with kidney protective effects as described above should be added. Volume expansion as seen in those with nephrotic syndrome should be treated with salt restriction and diuretic therapy. In summary, blood pressure control is critical to maintaining renal function. It should be followed closely and treated aggressively to delay CKD progression and reduce cardiovascular risk.

Cross-References

- ▶ Autoimmune Kidney Disease and Pregnancy
- ▶ IgA Nephropathy
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Scleroderma Renal Crisis
- ▶ Vasculitis and the Kidney

References

Bakris GL, Smith A. Effects of sodium intake on albumin excretion in patients with diabetic nephropathy treated with long-acting calcium antagonists. Ann Intern Med. 1996;125(3):201–4.

Bakris GL, Weir MR. Angiotensin-converting enzyme inhibitor-associated elevations in serum creatinine: is this a cause for concern? Arch Intern Med. 2000;160(5):685–93.

Bakris GL, Copley JB, et al. Calcium channel blockers versus other antihypertensive therapies on progression of NIDDM associated nephropathy. Kidney Int. 1996;50(5):1641–50.

Bakris GL, Mangrum A, et al. Effect of calcium channel or beta-blockade on the progression of diabetic nephropathy in African Americans. Hypertension. 1997;29(3):744–50.

Bakris GL, Weir MR, et al. Differential effects of calcium antagonist subclasses on markers of nephropathy progression. Kidney Int. 2004;65(6):1991–2002.

Benigni A, Tomasoni S, et al. Blocking angiotensin II synthesis/activity preserves glomerular nephrin in rats with severe nephrosis. J Am Soc Nephrol. 2001;12(5):941–8.

Bonnet F, Cooper ME, et al. Irbesartan normalises the deficiency in glomerular nephrin expression in a model of diabetes and hypertension. Diabetologia. 2001;44(7):874–7.

Brenner BM, Cooper ME, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med. 2001;345(12):861–9.

Brewster UC, Perazella MA. The renin-angiotensin-aldosterone system and the kidney: effects on kidney disease. Am J Med. 2004;116(4):263–72.

Brilla CG. Aldosterone and myocardial fibrosis in heart failure. Herz. 2000;25(3):299–306.

Brown C, Haddad N, Hebert L. Retarding progression of kidney disease. In: Floege J, Johnson RJ, Feehally J, editors. Comprehensive clinical nephrology. Philidelphia: Elsevier; 2010. p. 919–26.

Chobanian AV, Bakris GL, Cushman WC, Green LA, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: JNC-7. JAMA. 2003;289:2560–72.

Chrysostomou A, Becker G. Spironolactone in addition to ACE inhibition to reduce proteinuria in patients with chronic renal disease. N Engl J Med. 2001;345(12):925–6.

Durvasula RV, Petermann AT, et al. Activation of a local tissue angiotensin system in podocytes by mechanical strain. Kidney Int. 2004;65(1):30–9.

Fogo AB. Progression versus regression of chronic kidney disease. Nephrol Dial Transplant. 2006;21(2):281–4.

Gashti CN, Bakris GL. The role of calcium antagonists in chronic kidney disease. Curr Opin Nephrol Hypertens. 2004;13(2):155–61.

Heeg JE, de Jong PE, et al. Efficacy and variability of the antiproteinuric effect of ACE inhibition by lisinopril. Kidney Int. 1989;36(2):272–9.

Hollenberg NK. Aldosterone in the development and progression of renal injury. Kidney Int. 2004;66(1):1–9.

Hou FF, Xie D, et al. Renoprotection of Optimal Antiproteinuric Doses (ROAD) Study: a randomized controlled study of benazepril and losartan in chronic renal insufficiency. J Am Soc Nephrol. 2007;18(6):1889–98.

Hummel D, Raff U, et al. Dihydropyridine calcium antagonists are associated with increased albuminuria in treatment-resistant hypertensives. J Nephrol. 2010;23(5):563–8.

Jones-Burton C, Mishra SI, et al. An in-depth review of the evidence linking dietary salt intake and progression of chronic kidney disease. Am J Nephrol. 2006;26(3):268–75.

Levey A, Rocco M, et al. K/DOQI clinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. Am J Kidney Dis. 2004; 43(5 Suppl 1): S1–290.

Kincaid-Smith P, Fairley K, et al. Randomized controlled crossover study of the effect on proteinuria and blood pressure of adding an angiotensin II receptor antagonist to an angiotensin converting enzyme inhibitor in normotensive patients with chronic renal disease and proteinuria. Nephrol Dial Transplant. 2002;17(4):597–601.

Klahr S, Levey AS, et al. The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. N Engl J Med. 1994;330(13):877–84.

Lewis EJ, Hunsicker LG, et al. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med. 1993;329(20):1456–62.

Lewis EJ, Hunsicker LG, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med. 2001;345(12):851–60.

Luno J, Barrio V, et al. Effects of dual blockade of the renin-angiotensin system in primary proteinuric nephropathies. Kidney Int. 2002;62(Suppl 82): S47–52.

MacFadyen RJ, Lee AF, et al. How often are angiotensin II and aldosterone concentrations raised during chronic ACE inhibitor treatment in cardiac failure? Heart. 1999;82(1):57–61.

MacKinnon M, Shurraw S, et al. Combination therapy with an angiotensin receptor blocker and an ACE inhibitor in proteinuric renal disease: a systematic review of the efficacy and safety data. Am J Kidney Dis. 2006;48(1):8–20.

Maschio G, Alberti D, et al. Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. N Engl J Med. 1996;334(15):939–45.

Messerli FH, Bangalore S, et al. Risk/benefit assessment of beta-blockers and diuretics precludes their use for first-line therapy in hypertension. Circulation. 2008a;117(20):2706–15. discussion 2715.

Messerli FH, Bangalore S, et al. Telmisartan, ramipril, or both in patients at high risk of vascular events. N Engl J Med. 2008b;359(4):426–7. author reply 427.

Parving HH, Persson F, et al. Aliskiren combined with losartan in type 2 diabetes and nephropathy. N Engl J Med. 2008;358(23):2433–46.

Peterson D. http://ukidney.com/hypertension/no-benefit-of-aliskiren-added-on-to-ace-or-arb-a-disappointing-development. 2011.

Pfeffer MA, McMurray JJ, et al. Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. N Engl J Med. 2003;349(20):1893–906.

Pitt B, Zannad F, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med. 1999;341(10): 709–17.

Ruggenenti P, Perna A, et al. Effects of dihydropyridine calcium channel blockers, angiotensin-converting enzyme inhibition, and blood pressure control on chronic, nondiabetic nephropathies. Gruppo Italiano di Studi Epidemiologici in Nefrologia (GISEN). J Am Soc Nephrol. 1998;9(11):2096–101.

Ruggenenti P, Perna A, et al. Blood-pressure control for renoprotection in patients with non-diabetic chronic renal disease (REIN-2): multicentre, randomised controlled trial. Lancet. 2005;365(9463):939–46.

Ruiz-Ortega M, Ruperez M, et al. Angiotensin II: a key factor in the inflammatory and fibrotic response in kidney diseases. Nephrol Dial Transplant. 2006;21(1):16–20.

Sarafidis PA, Khosla N, et al. Antihypertensive therapy in the presence of proteinuria. Am J Kidney Dis. 2007;49(1):12–26.

Saruta T, Hayashi K, et al. Effects of candesartan and amlodipine on cardiovascular events in hypertensive patients with chronic kidney disease: subanalysis of the CASE-J Study. Hypertens Res. 2009;32(6):505–12.

Sato A, Hayashi K, et al. Effectiveness of aldosterone blockade in patients with diabetic nephropathy. Hypertension. 2003;41(1):64–8.

Struthers AD. Aldosterone escape during ACE inhibitor therapy in chronic heart failure. Eur Heart J. 1995;16(Suppl N):103–6.

Sun Y, Zhang J, et al. Local angiotensin II and transforming growth factor-beta1 in renal fibrosis of rats. Hypertension. 2000;35(5):1078–84.

The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal

failure in proteinuric, non-diabetic nephropathy. Lancet. 1997;349(9069):1857–63.
Tsouli SG, Liberopoulos EN, et al. Combined treatment with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers: a review of the current evidence. J Cardiovasc Pharmacol Ther. 2006;11(1):1–15.
Vogt L, Waanders F, et al. Effects of dietary sodium and hydrochlorothiazide on the antiproteinuric efficacy of losartan. J Am Soc Nephrol. 2008;19(5):999–1007.
Weir MR, Chrysant SG, et al. Influence of race and dietary salt on the antihypertensive efficacy of an angiotensin-converting enzyme inhibitor or a calcium channel antagonist in salt-sensitive hypertensives. Hypertension. 1998;31(5):1088–96.
Wolf G, Ritz E. Combination therapy with ACE inhibitors and angiotensin II receptor blockers to halt progression of chronic renal disease: pathophysiology and indications. Kidney Int. 2005;67(3):799–812.
Wright Jr JT, Bakris G, et al. Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. JAMA. 2002;288(19):2421–31.
Wuhl E, Trivelli A, et al. Strict blood-pressure control and progression of renal failure in children. N Engl J Med. 2009;361(17):1639–50.
Yoshioka T, Rennke HG, et al. Role of abnormally high transmural pressure in the permselectivity defect of glomerular capillary wall: a study in early passive Heymann nephritis. Circ Res. 1987;61(4):531–8.
Zannad F, McMurray JJ, et al. Eplerenone in patients with systolic heart failure and mild symptoms. N Engl J Med. 2011;364(1):11–21.

Psoriasis

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Synonyms

Psoriasis vulgaris

Definition

Psoriasis is an inflammatory, immune-mediated disease which can involve the skin, nails, and joints. Its occurrence may be influenced by environmental factors (Menter et al. 2008).

Introduction

Psoriasis is an inflammatory, immune-mediated disease that can affect a variety of skin surfaces, joints, and nails. Currently, there are several treatments available for the disease. Modalities include topical treatments, systemic medications, and phototherapy treatment. Most recently, biologic treatments have been used which work through the immune system to provide therapeutic benefit.

Psoriasis was first described by Robert Willan in 1809. In 1841, Hebra distinguished psoriasis from leprosy. Toward the end of the nineteenth century, Heinrich Koebner depicted psoriatic plaques emerging in areas of trauma (van de Kerkhof and Schalkwijk 2008).

Epidemiology

Generally, psoriasis affects about 2 % of the population (Menter et al. 2008). Worldwide, the occurrence of psoriasis varies based on age and geographic location. In adults, the prevalence of psoriasis ranges from 0.91 % in the United States to 8.5 % in Norway. In children, the prevalence is between 0 % in Taiwan to 2.1 % in Italy. The occurrence of psoriasis is more frequent in nations further from the equator (Parisi et al. 2013).

The occurrence of psoriasis may take place at any age. There are two peaks in incidence of psoriasis; the first peak occurs in early adulthood and the second peak occurs between ages 50 and 60 (Cohen et al. 2012).

Pathophysiology/Immunology

Traditionally, psoriasis had been classified as a T-helper (Th)-1 cell-mediated disease (Cai et al. 2012). Observations in support of this included elevated numbers of CD4+ TH1 and CD8+ cytotoxic cells type 1 (Tc1) and increased levels of cytokines including interferon-gamma (IFN-gamma), tumor necrosis factor (TNF)-alpha, and interleukin (IL)-12 (Cai et al. 2012).

More recently, it has been observed that in psoriasis, there is expansion and stimulation of not only Th-1 cells but also Th-17 and Th-22 cell populations (Reich 2012). In turn, there is release of TNF-alpha, IL-17, and IL-22 (Reich 2012).

The IL-23/Th-17 pathway is critical in psoriasis pathogenesis. This pathway facilitates the inflammatory response of the skin by directing cytokine and chemoattractant secretion and cytokine production (Monteleone et al. 2011). IL-23, a heterodimeric cytokine, consists of subunits IL-23p19 and IL-12p40 and is a crucial cytokine necessary for Th-17 cell maintenance and development (Cai et al. 2012). In psoriatic lesional skin and circulation, observations of increased Th-17 cells and related downstream molecules including IL-17A, IL-17F, IL-22, IL-21, and TNF-alpha have been noted (Cai et al. 2012).

Presentation

Skin

Classically, psoriasis presents with erythematous distinct scaly plaques overlying knees and elbows. The scale is a micaceous silvery scale. When the scale is rubbed or picked off, bleeding can occur. This is also known as Auspitz sign. In addition, lesions can occur in sites of trauma. Lesions occur between 10 days and a few weeks after the injury (Cohen et al. 2012). This is known as the Koebner or isomorphic phenomenon. Psoriasis of the skin can also involve the scalp, the intergluteal cleft, the palms and soles, the groin, and axillary regions.

Different types of psoriasis exist, but many individuals can have multiple subtypes that overlap (Menter et al. 2008) (Table 1).

Psoriasis, Table 1 Subtypes of psoriasis

Subtype of psoriasis	Description
Plaque psoriasis	The most common form of psoriasis
	Well-demarcated, red plaques ranging in size from 1 cm and beyond with overlying micaceous scale
	Favors the extremities (elbows and knees), scalp, trunk, and buttocks (James et al. 2005; Menter et al. 2008)
Sebopsoriasis	Features overlap with seborrheic dermatitis
	Seen on the scalp, face, breasts, flexural surfaces, and axilla (James et al. 2005)
Inverse psoriasis	Seen mostly in folds such as axilla and groin (James et al. 2005; Menter et al. 2008)
Guttate psoriasis	Small drop-like lesions, ranging in size between 1 and 10 mm, scattered mostly on the trunk, may occur after streptococcal infection
	Often seen in individuals less than 30 years old
	Responds well to phototherapy (James et al. 2005; Menter et al. 2008)
Pustular psoriasis	May be generalized or localized
	The localized form consists of erythema and pustules occurring on the palms and soles
	The acute generalized form, also known as von Zumbusch type, is characterized by a background of erythema with overlying pustules and fevers and toxicity
	May occur after administration of systemic corticosteroids is complete
	Treatment of choice is acitretin (James et al. 2005; Menter et al. 2008)
Impetigo herpetiformis	This is a form of pustular psoriasis seen in pregnancy
	Treatment may include delivery of the baby or systemic corticosteroids (James et al. 2005)
Erythrodermic psoriasis	Characterized by generalized erythema
	Involves most of the body surface area
	Chills, hypothermia, fluid loss may occur due to altered thermoregulation
	Fevers and fatigue may be seen (James et al. 2005; Menter et al. 2008)

Nails

Up to 45 % of psoriasis patients and almost 90 % of patients with psoriatic arthritis may have nail manifestations of psoriasis (Tan et al. 2012). About 5 % of patients present with nail psoriasis and have no skin manifestations of the disease (Tan et al. 2012).

Patients with nail psoriasis may have a variety of clinical findings including onychodystrophy, subungual hyperkeratosis, discoloration of the nail bed, onycholysis, and pitting of the nail plate. The most common nail finding in psoriasis is pitting. Splinter hemorrhages, leukonychia, red spots in the lunula, and crumbling of the nail plate may also be seen (Tan et al. 2012).

Joints

Psoriatic arthritis may occur with or without skin involvement. In addition, psoriatic arthritis may be accompanied solely by nail or scalp lesions.

Psoriasis, Table 2 Subtypes of psoriatic arthritis

Subtypes of psoriatic arthritis	Characteristics
Assymetric oligoarthritis	Common pattern, seen in up to half of male patients
	Initially affects digits causing a "sausage" appearance
	At any given time, less than five joints are involved (Franks 2004)
Symmetric polyarthritis	Most common subtype in women
	Rheumatoid-like pattern with involvement of hands, feet, and ankles
	Distinguished by involvement of the distal interphalangeal (DIP) joint (Franks 2004)
DIP joint	Symmetric involvement of the joint occurs in fewer than 5 % patients
	Mostly seen in men
	When involvement is asymmetric may overlap with oligoarthritic or rheumatoid-like types
	Nail involvement may occur (Franks 2004)
Arthritis mutilans	Osteolysis or resorption of bone with loss of joint space may occur leading to "pencil-in-cup" deformity which may be observed in radiographs (Franks 2004)
Spondylitis	Involvement of the neck, thoracic, and lumbosacral spine may occur
	Asymmetric sacroiliitis is seen (Franks 2004)

Generally, skin involvement occurs before joint involvement (Franks 2004) (Table 2).

Enthesopathy may be seen by itself or in conjunction with another subtype of psoriatic arthritis. In diagnosing psoriatic arthritis, it is important to establish the absence of rheumatoid factor. However, some patients may have both psoriatic arthritis and rheumatoid arthritis or may be part of the 5 % of the general population which is seropositive (Franks 2004).

Associations

Factors Which Exacerbate Psoriasis

Psoriasis may be exacerbated by a variety of factors. These include stress, high alcohol consumption, cold weather, skin trauma, and medications. Certain medications may exacerbate existing psoriatic disease, induce psoriatic lesions in patients with a history of psoriasis on skin that was previously uninvolved, or trigger new onset psoriasis (Dika et al. 2006). Drugs that are highly implicated include beta blockers, lithium, synthetic antimalarials, nonsteroidal anti-inflammatory drugs (NSAIDs), and tetracyclines. Medications that may possibly exacerbate psoriasis include ACE-inhibitors, terbinafine, and interferons (Dika et al. 2006).

Psoriasis and Metabolic Syndrome

There appear to be associations between psoriasis, psoriatic arthritis, cardiovascular disease, obesity diabetes, and non-alcoholic fatty liver disease. These associations have implications in terms of potential screening in patients with psoriasis and psoriatic arthritis (Johnsson et al. 2012). The prevalence of the metabolic syndrome in US adults with psoriasis is higher than the prevalence of metabolic syndrome in US adults without psoriasis; 40 % of psoriatics were found to have the metabolic syndrome versus 23 % of non-psoriatics who were determined to have the metabolic syndrome (Love et al. 2011).

Psoriasis and Infection

Guttate psoriasis has been linked to streptococcal infection. Specifically, it has been noted that tonsillar Streptococcus pyogenes infection may precede the onset of guttate psoriasis (Fry and Baker 2007). Colonization of the skin and/or gut with Staphylococcus aureus, Malassezia, and Candida albicans has been associated with disease exacerbation (Fry and Baker 2007).

HIV-associated psoriasis may be severe and difficult to treat. The severity of psoriasis in a patient with HIV may vary with the degree of immunosuppression. Guttate, inverse, and erythrodermic psoriasis are the most common subtypes of psoriasis in patients with HIV. In this population, psoriatic arthritis is more frequent and has greater severity. Treatment of psoriasis in HIV patients may be challenging as therapeutic choices may be limited and have the ability to cause more complications (Menon et al. 2010).

Diagnosis

Psoriasis may be diagnosed on the basis of patient history and clinical presentation. Biopsies of the skin may be done for confirmation of skin disease. X-rays may be helpful in diagnosing psoriatic arthritis.

The histology of psoriasis reveals confluent parakeratosis, Munro microabscesses, which consist of neutrophils in the stratum corneum, and spongiform pustules of Kogoj which are neutrophils in the stratum spinosum. Additional features include epidermal thinning above dermal papillae, hypogranulosis, acanthosis, dilation of capillaries in dermal papillae, and lymphocytes located perivascularly (Rapini 2005).

Treatment and Management

There are many treatments for psoriasis. Appropriate therapy depends on type and extent of disease. Patient comorbidities also affect the choice of therapy.

Topical and Local Treatments

For limited localized disease, topical therapy is often the first line of treatment. Topical agents can be used in mild to moderate disease. They can also be used in conjunction with ultraviolet (UV) light or systemic agents in resistant lesions. There are several different types of vehicles available. The vehicle can be adjusted based on body surface affected and patient preference. Topical agents may also be used in combination (Menter et al. 2009a).

Topical corticosteroids are frequently the mainstay of therapy in psoriatic patients. Topical steroids are recommended in psoriasis that involves less than or equal to 10 % body surface area (Paul et al. 2012). They are vasoconstrictive, immunosuppressive, antiproliferative, and anti-inflammatory (Menter et al. 2009a). Corticosteroids bind to intracellular receptors and regulate the transcription of genes coding for inflammatory cytokines (Menter et al. 2009a).

Mid to high potency topical steroids may be used as induction therapy in adults with psoriatic lesions on the trunk and extremities. In infants and young children and in adults with lesions on the face and intertriginous areas, lower potency topical steroids are used (Menter et al. 2009a). Side effects of topical corticosteroids generally occur only locally. Telangiectasias, striae, atrophy of the skin, purpura, ecchymosis, and acneiform eruptions can occur. Systemic side effects are very uncommon but are more of a risk with use of high and ultrahigh potency topical corticosteroids. Concerns include cataracts, glaucoma, Cushing's syndrome, and osteonecrosis of the femoral head and hypothalamic-pituitary-adrenal axis suppression (Menter et al. 2009a). Intralesional corticosteroid treatment may be used for thicker psoriatic plaques and has a similar side effect profile to ultrahigh potency topical steroid therapy.

Vitamin D analogues such as calcipotriene are believed to bind to vitamin D receptors inhibiting the proliferation of keratinocytes and promoting the differentiation of keratinocytes. They may be used in conjunction with topical corticosteroids in the induction phase of treatment and may be used as monotherapy in the maintenance phase of treatment (Menter et al. 2009a). As with topical corticosteroids, local side effects are more common. Redness, itch, edema, burning, peeling, and xerosis may occur. Systemic side effects are rare but can potentially include hypercalcemia and suppression of parathyroid hormone. UVA inactivates calcipotriene so patients are advised to apply the medication after exposure (Menter et al. 2009a).

Calcineurin inhibitors are another topical option. They include tacrolimus and pimecrolimus. They inhibit inflammatory cytokine synthesis and are particularly helpful in the treatment of psoriasis on the face and intertriginous areas since they are not known to cause skin atrophy. In addition, they are helpful in the maintenance phase of treatment. Burning and pruritus are common side effects. There is a black box warning on these medications due to potential risk of malignancies and deficient long-term safety data (Menter et al. 2009a).

Other topical options include the combination of calcipotriene and betamethasone propionate ointment, salicylic acid, anthralin, and coal tar.

Tazarotene and salicylic acid are helpful in treating resistant plaques (Paul et al. 2012).

Phototherapy

Phototherapy is indicated for patients with involvement of large body surface areas, mostly located on the trunk and the extremities. The options for phototherapy include PUVA (psoralen + UVA light) and NB-UVB (narrowband UVB light). PUVA is more effective. However, NB-UVB is preferred due to convenience and the increased risk of skin cancer associated with PUVA. It is recommended that patients undergoing phototherapy use ocular protection to prevent cataracts and use facial protection to prevent photoaging and lentigines. Phototherapy sessions should take place 2–3 times per week. Usually, clearance of psoriatic lesions occurs between 20 and 30 treatments (Paul et al. 2012).

Systemic Medications

There are many systemic therapies available for the treatment of psoriasis. Treatment choice depends on the type and extent of psoriasis. Patient comorbidities and characteristics affect the choice of therapy as well.

Methotrexate has been used in the treatment of psoriasis for several years. It is used in both severe psoriasis and psoriatic arthritis. It inhibits dihydrofolate reductase which reduces tetrahydrofolate synthesis. Tetrahydrofolate is a cofactor necessary in the synthesis of DNA and RNA. The effects of methotrexate in psoriasis are largely believed to be immunosuppressive; it inhibits the proliferation of lymphoid tissue (Callen et al. 2007). Methotrexate is administered once weekly either orally or parenterally. Prior to therapy, a test dose may be given to check for bone marrow suppression. Weekly dosages range between 7.5 and 25 mg (Menter et al. 2009b). Initial clinical response occurs within 4 weeks of initiating therapy, while full therapeutic effects often take between 2 and 3 months to occur (Callen et al. 2007). Folate supplementation may be helpful in preventing gastrointestinal side effects and bone marrow toxicity. More common side effects include stomatitis, anorexia, nausea, and fatigue. Major side effects include myelosuppression, pancytopenia, pulmonary toxicity, hepatotoxicity, lymphomas with Epstein-Barr virus, and renal toxicity (Callen et al. 2007). Patients require careful lab monitoring and diligent follow-up of the liver. Methotrexate is contraindicated in pregnant and nursing women. Men and women should wait 3 months after stopping the medication prior to trying to conceive (Menter et al. 2009b).

Cyclosporine is used as a short-term medication for the clearance of severe recalcitrant psoriasis. It may be used in patients who have failed other treatments, are experiencing a new flare of disease, or need clearance for a short period of time for a life event. Cyclosporine may be helpful in erythrodermic or pustular psoriasis, as well. Cyclosporine inhibits interleukin-2 (IL-2) produced by T lymphocytes through the inhibition of calcineurin by way of a complex formed between cyclosporine and cyclophilin. Decreased IL-2 production causes reduced numbers of CD4 and CD8 cells (Lee and Koo 2007). It is dosed between 2.5 and 5 mg/kg/day. Adverse effects include renal toxicity and hypertension. In addition, tremors, headaches, paresthesias, and hyperesthesias may be reported. Hypertrichosis and gingival hyperplasia may occur. Labs should be checked regularly as hyperkalemia, hyperuricemia, hypomagnesemia, and hyperlipidemia may develop. Gastrointestinal and musculoskeletal side effects may also occur. Cyclosporine is pregnancy category C (Lee and Koo 2007).

The retinoids are another group of oral medications which are used in the treatment of psoriasis. The exact mechanism of retinoids in psoriasis has not been elucidated. However, this group of medications affects cell differentiation and growth. In addition, retinoids work against inflammation and may be immunomodulatory. Acitretin is currently the retinoid most commonly used in psoriasis. It may be used in chronic plaque psoriasis and pustular psoriasis (Menter et al. 2009b). It may also be effective in treating nails that are affected by psoriasis. Acitretin can be

dosed from 10 to 50 mg and higher once nightly. Acitretin is useful in treating severe psoriasis in patients with HIV. Common side effects include dry skin, dry eyes, cheilitis, epistaxis, alopecia, brittle nails, and sticky or burning skin. In addition, lipid abnormalities, particularly increased triglycerides, and increased liver function tests may be seen. Acitretin is teratogenic and generally should not be given to women with childbearing potential (Menter et al. 2009b). Alcohol should be avoided as ethanol converts acitretin to etretinate which has a half-life to 168 days. Combination treatment with acitretin and NBUVB or PUVA is even more effective (Menter et al. 2009b).

Other systemic options include azathioprine, fumarates, hydroxyurea, leflunomide, mycophenolate mofetil, sulfasalazine, tacrolimus, and 6-thioguanine (Menter et al. 2009b).

Biologic Medications

One of the newest groups of systemic medications used in the treatment of psoriasis are the biologics. There are different types of biologic therapies, but they all target the immune system. Baseline screening includes complete blood cell count, liver function tests, hepatitis panel, and screening for tuberculosis (Kim et al. 2012).

The TNF-alpha inhibitors include etanercept, adalimumab, and infliximab. T cells and keratinocytes are sources of TNF-alpha which plays a crucial role as an inflammatory cytokine in psoriasis. A transmembrane-bound precursor undergoes cleavage to yield TNF-alpha which subsequently binds TNF-alpha receptor. TNF-alpha inhibitors block this pathway thereby obstructing the inflammatory cascade (Laws and Young 2012). These medications increase the risk of infection, particularly upper respiratory infections. Other risks include lupus-like syndrome from autoantibody formation and worsening of demyelinating diseases. Other potential associated adverse effects include increased mortality in patients with congestive heart failure, risk for the development of lymphomas, and non-melanoma skin cancers. Causality of these adverse effects has not been established (Kim et al. 2012).

Etanercept, a fusion protein made up of a TNF-alpha receptor fused to the Fc portion of human immunoglobulin G (IgG), is used in psoriasis and psoriatic arthritis. It is dosed at 50 mg subcutaneously once or twice a week for 3 months. Subsequently, patients receive 50 mg subcutaneously once a week. Injection site reactions can occur (Kim et al. 2012).

Adalimumab, a recombinant human immunoglobulin monoclonal antibody with high-affinity binding to TNF-alpha, is a therapeutic option in psoriasis and psoriatic arthritis. A loading dose of 80 mg is given subcutaneously. This is followed by subcutaneous doses of 40 mg every other week (Kim et al. 2012).

Infliximab, a chimeric monoclonal antibody which binds human TNF-alpha, is used to treat psoriasis and psoriatic arthritis. Intravenous infusions of the medication dosed at 5 mg/kg are administered at weeks 0, 2, and 6. Subsequently, the medication is given every 8 weeks. Rarely, anaphylaxis-like infusion reaction can occur (Kim et al. 2012).

Recombinant DNA technology has been employed to obtain alefacept, a human fusion protein which binds to memory effector T cells via CD2. Through this mechanism, alefacept obstructs the activation of T cells. Alefacept is used to treat psoriasis and administered as a 15 mg intramuscular injection every week for 12 weeks. Subsequently, there is a medication-free interval of 12 weeks (Kim et al. 2012).

Ustekinumab is a monoclonal antibody to p40, a subunit of both IL-12 and IL-23. IL-12 is believed to play a role in the differentiation of T cells to T-helper lymphocyte (Th)-1 cells, while IL-23 is thought to promote the differentiation of naive T cells toward Th-17 cells. This medication is used to manage psoriasis and is dosed at 45 mg subcutaneously at weeks 0, 4, and subsequently every 12 weeks. Patients who weigh more than 100 kg may be given 90 mg subcutaneously instead (Laws and Young 2012; Kim et al. 2012).

Conclusion

Psoriasis, an inflammatory, immune-mediated disease, can affect a variety of skin surfaces, joints, and nails. There has been much new research regarding the immunopathogenesis of this disease. This has resulted in the introduction of many new therapies with the promise of many more to come in the future.

References

Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. Cell Mol Immunol. 2012;9:302–9.

Callen JP, Kulp-Shorten CL, Wolverton SE. Methotrexate. In: Wolverton SE, editor. Comprehensive dermatologic drug therapy. 2nd ed. Philadelphia: Saunders; 2007. p. 163–82.

Cohen SN, Baron SE, Archer CB, et al. Guidance on the diagnosis and clinical management of psoriasis. Clin Exp Dermatol. 2012;37(Suppl 1):13–8.

Dika E, Varotti C, Bardazzi F, Maibach HI. Drug-induced psoriasis: an evidence based overview and the introduction of psoriatic drug eruption probability score. Cutan Ocul Toxicol. 2006;25:1–11.

Franks Jr AG. Psoriatic arthritis and Reiter syndrome. In: Sontheimer RD, Provost TT, editors. Cutaneous manifestations of rheumatic diseases. 2nd ed. Philadelphia: Lippincott Williams and Wilkins; 2004. p. 197–204.

Fry L, Baker BS. Triggering psoriasis: the role of infections and medications. Clin Dermatol. 2007;25:606–15.

James W, Berger T, Elston D. Seborrheic dermatitis, psoriasis, recalcitrant palmoplantar eruptions, pustular dermatitis and erythroderma. In: Andrews' diseases of the skin: clinical dermatology. 10th ed. Philadelphia: Saunders; 2005. p. 191–205.

Johnsson H, McInnes IB, Sattar N. Cardiovascular and metabolic risks in psoriasis and psoriatic arthritis: pragmatic clinical management based on available evidence. Ann Rheum Dis. 2012;71:480–3.

Kim IH, West CE, Kwatra SG, Feldman SR, O'Neill JL. Comparative efficacy of biologics in psoriasis: a review. Am J Clin Dermatol. 2012;13:365–74.

Laws PM, Young HS. Current and emerging systemic treatment strategies for psoriasis. Drugs. 2012;72: 1867–80.

Lee CS, Koo JYM. Cyclosporine. In: Wolverton SE, editor. Comprehensive dermatologic drug therapy. 2nd ed. Philadelphia: Saunders; 2007. p. 219–38.

Love TJ, Qureshi AA, Karlson EW, Gelfand JM, Choi HK. Prevalence of the metabolic syndrome in psoriasis: results from the national health and nutrition examination survey, 2003–2006. Arch Dermatol. 2011;147:419–24.

Menon K, Van Voorhees AS, Bebo Jr BF, Gladman DD, Hsu S, Kalb RE, Lebwohl MG, Strober BE, et al. Psoriasis in patients with HIV infection: from the medical board of the national psoriasis foundation. J Am Acad Dermatol. 2010;62:291–9.

Menter A, Gottlieb A, Feldman SR, Van Voorhees AS, Leonardi CL, Gordon KB, Lebwohl M, Koo JYM, Elmets CA, Korman NJ, Beutner KR, Bhushan R. Guidelines of care for the management of psoriasis and psoriatic arthritis: section 1. Overview of psoriasis and guidelines of care for the treatment of psoriasis with biologics. J Am Acad Dermatol. 2008;58:826–50.

Menter A, Korman NJ, Elmets CA, Feldman SR, Gelfand JM, Gordon KB, Gottlieb A, Koo JYM, Lebwohl M, Lim HW, Van Voorhees AS, Beutner KR, Bhushan R. Guidelines of care for the management of psoriasis and psoriatic arthritis: section 3. Guidelines of care for the management and treatment of psoriasis with topical therapies. J Am Acad Dermatol. 2009a;60:643–59(A).

Menter A, Korman NJ, Elmets CA, Feldman SR, Gelfand JM, Gordon KB, Gottlieb AB, Koo JY, Lebwohl M, Lim HW, Van Voorhees AS, Beutner KR, Bhushan R. Guidelines of care for the management of psoriasis and psoriatic arthritis: section 4. Guidelines of care for the management and treatment of psoriasis with traditional systemic agents. J Am Acad Dermatol. 2009b;61:451–85(B).

Monteleone G, Pallone F, MacDonald TT, Chimenti S, Costanzo A. Psoriasis: from pathogenesis to novel therapeutic approaches. Clin Sci. 2011;120:1–11.

Parisi R, Symmons DP, Griffiths CE, Ashcroft DM, et al. Global epidemiology of psoriasis: a systematic review of incidence and prevalence. J Invest Dermatol. 2013; 133: 377–85.

Paul C, Gallini A, Archier E, Castela E, Devaux S, Aractingi S, Aubin F, Bachelez H, Cribier B, Joly P, Jullien D, Le Maître M, Misery L, Richard MA, Ortonne JP. Evidence-based recommendations on topical treatment and phototherapy of psoriasis: systematic review and expert opinion of a panel of dermatologists. J Eur Acad Dermatol Venereol. 2012;26(Suppl 3):1–10.

Rapini RP. Eczematous and papulosquamous diseases. In: Rapini RP, editor. Practical dermatopathology. Philadelphia: Mosby Elsevier; 2005. p. 45–59.

Reich K. The concept of psoriasis as a systemic inflammation: implications for disease management. J Eur Acad Dermatol Venereol. 2012;26:3–11.

Tan ES, Chong WS, Tey HL. Nail psoriasis: a review. Am J Clin Dermatol. 2012;13:375–88.

van de Kerkhof PCM, Schalkwijk J. Psoriasis. In: Bolognia JL, Jorizzo JL, Rapini RP, editors. Dermatology. 2nd ed. Philadelphia: Mosby Elsevier; 2008.

PTPN22

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Synonyms

Lymphoid phosphatase, LYP; Lymphoid-specific protein tyrosine phosphatase; PEST-domain phosphatase; PEST-enriched phosphatase, Pep; Protein tyrosine phosphatase, non-receptor type 8; Tyrosine-protein phosphatase non-receptor type 22

Definition

PTPN22 – a gene encoding the lymphoid phosphatase, a classical non-receptor protein tyrosine phosphatase expressed in hematopoietic cells.

Discovery of *PTPN22*

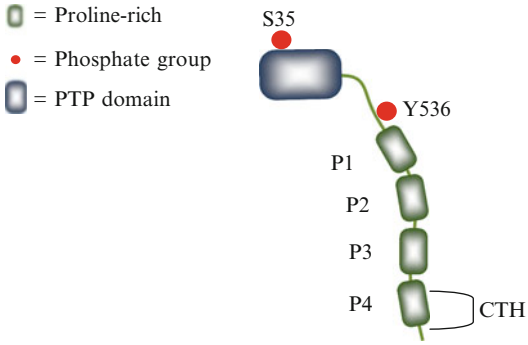
The human *PTPN22* gene is located on chromosome 1p13.3–13.1 and encodes the lymphoid phosphatase (LYP), a protein tyrosine phosphatase (PTP) cloned in 1999 by Chaim Roifman's laboratory (Stanford et al. 2010; Veillette et al. 2009). LYP shares 70 % sequence identity with the orthologous murine phosphatase Pep (PEST-enriched phosphatase), which had been cloned in 1992 by Matthew Thomas' laboratory and originally identified under the gene name *Ptpn8*. LYP/Pep belong to the PEST-enriched subfamily of non-receptor classical PTPs, which includes PTP-PEST (encoded by the *PTPN12* gene) and the brain-derived phosphatase 1 (BDP1, also known as the hematopoietic stem cell fraction phosphatase, PTP-HSCF, encoded by the *PTPN18* gene). As shown in Fig. 1, LYP and Pep are 105 kDa proteins containing three distinct regions: an N-terminal PTP catalytic domain, an interdomain region, and a C-terminal domain with four proline-rich motifs, termed P1–P4. The P4 motif is located within a carboxy-terminal homology (CTH) domain that is also found in BDP1 and PTP-PEST. Crystal structures of the catalytic domain of LYP have been solved and reveal a loop specific to the PEST-enriched PTPs. This region (amino acids 35–42) resides between the α1 and α2′ helices of the active site and participates in substrate binding (Orru et al. 2009; Yu et al. 2007).

LYP Expression in the Immune System

LYP/Pep is expressed only in hematopoietic cells. Three splice variants have been reported, designated LYP1, LYP2, and LYP3 (Burn et al. 2011). Additional shorter isoforms of LYP resulting from alternative splicing have been documented. Thus far, all functional studies have been performed on LYP1 (also designated LYP), the longest isoform of 807 amino acids. LYP2 was identified in resting T cells and contains a shorter C-terminus, which lacks the P2–P4 motifs and contains a unique tail of seven amino acids. LYP3 has a 28-amino acid deletion between the P1 and P2 motifs. No detailed expression surveys of the shorter isoforms, nor studies of putative shorter variants of Pep, have been reported. LYP1 is found in all hematopoietic cell lineages. At the mRNA level, the highest expression is found in natural killer cells and neutrophils, with lower expression in $CD8^+$ T cells, and the lowest in monocytes, dendritic cells (DCs), and $CD4^+$ T cells and B cells (Begovich et al. 2004). LYP/Pep is found in the cytoplasm, although Pep has been reported to localize to the nucleus and plasma membrane (Burn et al. 2011; Stanford et al. 2010; Veillette et al. 2009).

LYP Function in T Cells

The majority of studies on the function of LYP/Pep have been conducted in T cells (Stanford et al. 2010; Veillette et al. 2009). As shown in Fig. 2, LYP/Pep function as potent negative regulators of T cell activation through inhibition of key mediators of signal transduction downstream the T cell receptor (TCR).

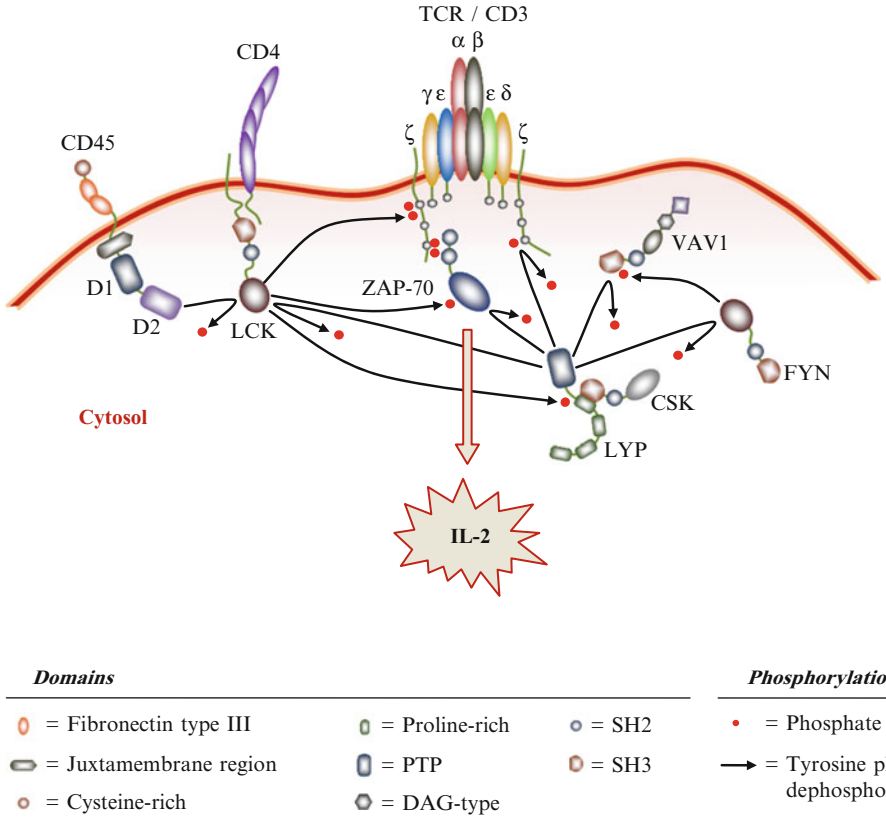


PTPN22, Fig. 1 Schematic representation of the LYP protein. LYP contains an N-terminal catalytic PTP domain, an interdomain region, and a C-terminal domain containing four proline-rich motifs, designated P1–P4, and a CTH domain. Reported sites of phosphorylation on serine (S) and tyrosine (Y) are shown

Through a combination of overexpression studies, substrate-trapping techniques, and studies using Pep-deficient mice, the substrates of LYP/Pep in T cells have been identified as the positive regulatory tyrosine residues in the activation loop of the Src family kinases (SFKs), lymphocyte-specific protein tyrosine kinase (LCK, Y394), and oncogene related to SRC, FGR, and YES (FYN, Y417) and the Syk family kinase zeta-chain (TCR)-associated protein kinase 70 kDa (ZAP-70, Y493); immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3ζ-chains; as well as phosphotyrosine residues in CD3ε, vav 1 guanine nucleotide exchange factor (Vav1), and valosin-containing protein (Vcp). Through dephosphorylation of these early mediators of signaling, LYP/Pep inhibits potentiation of the wave of tyrosine phosphorylation needed for early T cell activation and thus regulates signaling proximal to the TCR.

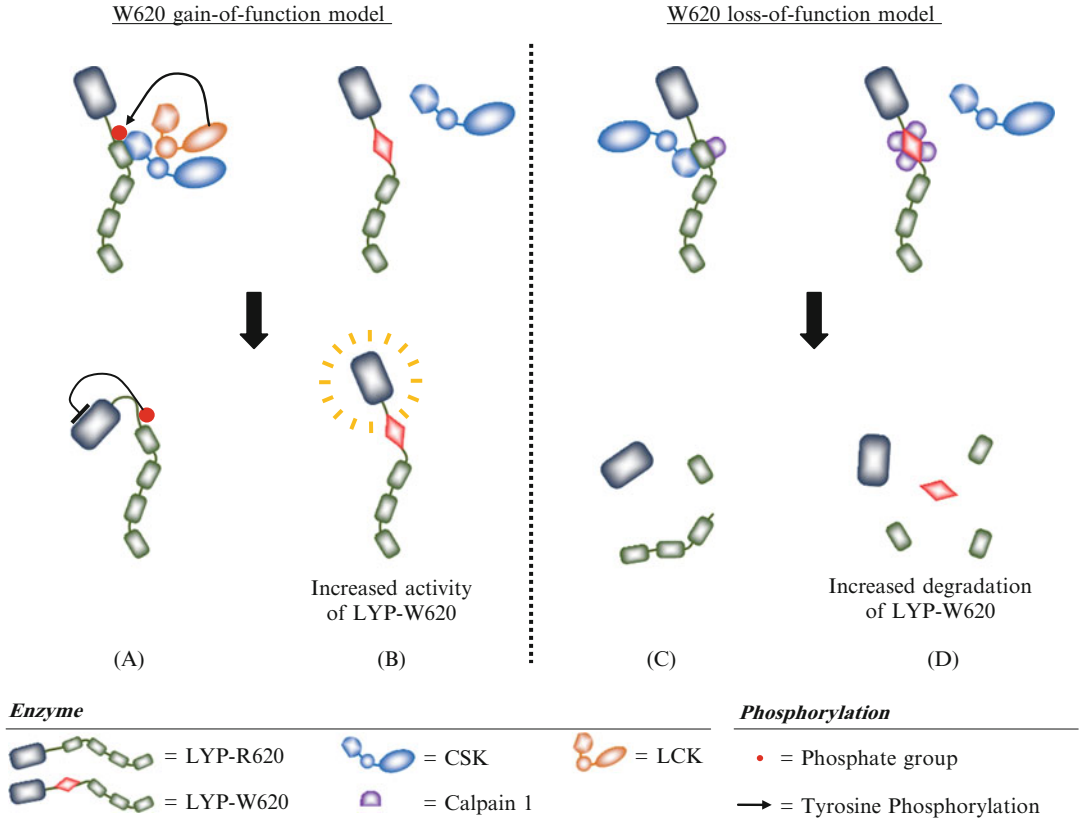
As demonstrated by Andrew Chan's group, in vivo deletion of Pep leads to increased T cell activation (Stanford et al. 2010; Veillette et al. 2009). T cells from *Ptpn22*$^{-/-}$ mice show enhanced TCR signaling, as evidenced by increased positive selection in the thymus, increased numbers of effector/memory CD4^{+} and CD8^{+} T cells, and increased responsiveness of effector/memory cells to TCR engagement. Naïve T cells from these mice show no difference in response to TCR stimulation, correlating with the expression pattern of LYP in T cells, which is high in thymocytes, low in naïve T cells, and high in mature and/or activated T cells. *Ptpn22*$^{-/-}$ mice also exhibit spontaneous germinal center formation in the spleen and Peyer's patches of aging mice, which have been attributed to an increase in T cell help. No spontaneous pathological phenotype has been found in these mice. Crossing of the *Ptpn22*$^{-/-}$ mouse with the CD45 E613R "wedge" model exacerbated the autoimmunity caused by the wedge mutation, indicating the importance of the threshold of lymphocyte signaling in maintaining immune homeostasis (Zikherman et al. 2009).

LYP contains four proline-rich motifs (P1–P4) that are potential binding sites for proteins containing SH3 domains. A well-documented interaction in T cells is that between the P1 motif of LYP/Pep and the SH3 domain of the negative regulatory tyrosine kinase C-terminal Src kinase (CSK) (Veillette et al. 2009). CSK is a potent inhibitor of TCR signaling through phosphorylation of the inhibitory tyrosine residue of the SFKs LCK and FYN. A proposed model for the function of the LYP-CSK complex is that it plays a dual role in controlling the threshold of TCR signaling. On one hand, the complex inhibits TCR signaling through synergistic repression of SFK activity by bringing LYP and CSK into close proximity to their SFK substrates. The phosphorylation of the inhibitory tyrosine by CSK, together with dephosphorylation of the activating phosphotyrosine by LYP, inactivates the SFKs and inhibits TCR signaling. On the other hand, it has been recently proposed that the complex between LYP and CSK also functions to sustain TCR signaling by tempering the activity of LYP upon TCR engagement. CSK recruits LCK to LYP, facilitating the phosphorylation of LYP by LCK on the inhibitory residue Y536 (see Fig. 3a). Phosphorylation on this site inhibits the enzymatic activity of LYP, allowing LCK to remain in an activated state to promote signaling through the TCR. According to this model, the strength and duration of TCR signaling is finely tuned by the feedback loop between LYP, CSK, and LCK to maintain T cells in the proper activation state (Fiorillo et al. 2010).



PTPN22, Fig. 2 LYP inhibits early TCR signaling. LYP acts as a gatekeeper of T cell activation through dephosphorylation of key mediators of signaling downstream the TCR. Upon ligand engagement of the TCR, a wave of tyrosine phosphorylation events occurs, which signals the response of the T cell. Key signaling mediators for this process are the SFKs LCK and FYN, the Syk family kinase ZAP-70, and downstream adaptor proteins. Upon TCR engagement, the SFKs become activated by phosphorylation in their activation motifs (Y394 of LCK and Y417 of FYN). The SFKs then phosphorylate the tandem tyrosine residues of the ITAMS of the TCR-associated CD3ζ-chain, which provide docking sites for the SH2 domains of ZAP-70. Once recruited to the TCR, ZAP-70 is phosphorylated and activated by LCK. ZAP-70 subsequently phosphorylates downstream adaptor proteins which nucleate signaling complexes to potentiate the wave of phosphorylation needed to transmit the TCR stimulation signal to the T cell nucleus. This signaling cascade leads to a myriad of responses by the T cell, which leads to T cell activation and, ultimately, production of IL-2, an autocrine growth factor for T cells. LYP inhibits this process through dephosphorylation of the activating tyrosine of the SFKs and ZAP-70, the ITAMs of the CD3ζ-chain, Vav1, and other mediators of signaling. Within this signaling cascade, LYP forms a high stoichiometry complex with CSK, a kinase that inhibits SFKs by phosphorylation on their negative regulatory tyrosine residue

Combined molecular and functional studies in T cells indicate that the activity of LYP is also regulated by intramolecular interactions and additional posttranslational modifications. Protein kinase C was shown by Zhong-Yin Zhang's laboratory to phosphorylate S35 in the LYP-specific loop of the catalytic domain, resulting in inhibition of the phosphatase activity (Yu et al. 2007). PTP-PEST is also inhibited by phosphorylation on the homologous residue, S39. Disulfide bond formation between the catalytic cysteine (C227) and a cysteine residue located outside the signature motif (C129) was reported to inhibit the phosphatase activity of LYP through a reversible oxidation mechanism. An intramolecular interaction between the catalytic



PTPN22, Fig. 3 Current models describing the molecular effect of the LYP-R620W polymorphism in T cells. (**a, b**) LYP-W620 gain-of-function model. (**a**) *Upper panel*, LYP-R620 binds CSK via the P1 motif of LYP-R620 and the SH3 domain of CSK. CSK recruits LCK to LYP-R620 through a currently unknown mechanism. LCK then phosphorylates LYP-R620 on Y536 in the interdomain region. *Lower panel*, phosphorylation of LYP-R620 at this site reduces the phosphatase activity of LYP-R620, leading to decreased inhibition of TCR signaling. (**b**) *Upper panel*, the W620 amino acid substitution in the P1 motif of LYP impairs the interaction between LYP and CSK. The CSK-dependent recruitment of LCK to LYP is reduced, and thus LYP-W620 undergoes less phosphorylation on the inhibitory Y536 residue. *Lower panel*, LYP-W620 is more enzymatically active, leading to gain-of-function inhibition of TCR signaling. (**c, d**) LYP-W620 loss-of-function model. (**c**) *Upper panel*, LYP-R620 binds to calpain through an unknown mechanism. *Lower panel*, binding of LYP to calpain leads to proteolysis of LYP. (**d**) *Upper panel*, LYP-W620 has increased interaction with calpain, possibly due to impaired interaction with CSK. *Lower panel*, LYP-W620 undergoes increased calpain-dependent proteolysis, leading to decreased expression of LYP-W620 and loss-of-function inhibition of TCR signaling

domain and the proximal interdomain region of LYP was also shown to regulate the enzymatic activity of the phosphatase (Stanford et al. 2010).

Little characterization of the regulation of the stability of LYP/Pep at the RNA or protein level has been reported. Pep expression was shown to be downregulated in T cells at the mRNA level by miRNA-181a (Veillette et al. 2009). At the protein level, LYP/Pep was reported to bind calpain, a Ca^{++}-dependent protease, through an interaction that promotes calpain- and proteasome-mediated degradation of the phosphatase (Zhang et al. 2011).

LYP Function in B Cells

Although LYP is highly expressed in other immune cell lineages, to date, little is known about its function outside of T cells. Several studies by Jane Buckner's laboratory in primary B cells from carriers of a functional variant of

LYP (R620W, see below) indicate that LYP inhibits human B cell receptor (BCR) signaling and possibly regulates selection of the B cell repertoire (Habib et al. 2012). Partial knockdown of Pep expression in an immature mouse B cell line was shown to inhibit BCR-triggered responses, suggesting a role for Pep as a positive regulator of BCR signaling in immature B cells. However, substrates of LYP/Pep in B cells have not yet been reported, and in vivo deletion of Pep showed no effect on BCR signaling. Whether this is due to compensation phenomena after knockdown/knockout of the phosphatase, to functional differences between the human and mouse proteins, or to another effect remains to be clarified (Veillette et al. 2009).

LYP Function in Myeloid Cells

A myeloid phenotype of the *Ptpn22*$^{-/-}$ mouse was recently reported (Obiri et al. 2012). This study indicates that Pep promotes mast cell degranulation through positive regulation of PLCγ1 and Ca^{++} signaling. Mice deficient in Pep showed impaired PLCϒ1 phosphorylation and Ca^{++} mobilization, defective mast cell degranulation, and decreased susceptibility to antigen-induced anaphylaxis. Additionally, a knock-in mouse carrying a functional variant of PTPN22 (Pep-W619) was reported to show increased numbers of splenic DCs, increased responsiveness of DCs to LPS stimulation, and increased T cell priming (Zhang et al. 2011). In humans, the presence of this same functional variant affects responsiveness of patients to imatinib treatment for chronic myeloid leukemia. These findings suggest a role for LYP in the signaling and/or function of myeloid cells, and further studies are warranted to understand the effect of LYP in these pathways and to determine if LYP is involved in homeostasis of the innate immune system.

PTPN22 in Human Disease

A role for *PTPN22* in human disease was identified in 2004 when a single-nucleotide polymorphism (SNP) in the gene, C1858T, was found by candidate gene approach to increase the risk of type 1 diabetes (T1D) in two independent populations (Bottini et al. 2004). That same year, two additional groups reported associations of the same SNP with rheumatoid arthritis (RA) (Begovich et al. 2004) and systemic lupus erythematosus (SLE) (Kyogoku et al. 2004). Since then, numerous studies have confirmed the T1858 allele to be a susceptibility locus for multiple, but not all, autoimmune diseases (Table 1) (Burn et al. 2011; Chung and Criswell 2007; Gregersen and Olsson 2009; Stanford et al. 2010). A smaller number of more recent studies have suggested that additional functional SNPs in the gene also regulate predisposition toward autoimmunity and have suggested a broader role for the *PTPN22* gene in regulating immune homeostasis. An overview of these polymorphisms, and their effect on human disease, is described below.

C1858T/R620W (rs2476601): This SNP encodes an R620W substitution in the P1 motif of LYP, disrupting the interaction of the phosphatase with CSK. The T1858 allele is a causal, population-independent risk factor for numerous organ-specific and systemic autoimmune diseases and has been identified as a general autoimmunity gene. The variant behaves as a codominant allele, conferring risk of disease in heterozygous carriers, and more so when present in both copies. The risk associated with the allele is variable among diseases and is the highest for RA and T1D. Indeed, *PTPN22* ranks the second most significant genetic risk factor for RA and the third most significant one for T1D. Interestingly, the T1858 allele does not increase risk for all autoimmune diseases and even acts as a protective variant for some, suggesting there may be shared pathogenic mechanisms among subsets of autoimmune diseases. There initially was reported no association with Crohn's disease, but recent studies have shown a protective effect, while there is no effect in ulcerative colitis (Burn et al. 2011; Chung and Criswell 2007; Gregersen and Olsson 2009; Stanford et al. 2010).

The T1858 allele also associates with increased risk of endometriosis, a disease characterized by autoimmune-like features, atherosclerosis, a disease with a strong inflammatory component, and bilateral Meniere's disease.

PTPN22, Table 1 ***PTPN22*** **associations with human autoimmunity.** Table denotes associations of *PTPN22* SNPs C1858T, G788A, and G-1123C with autoimmune diseases (Burn et al. 2011; Chung and Criswell 2007; Diaz-Gallo et al. 2011a, b; Gregersen and Olsson 2009; Stanford et al. 2010)

Autoimmune disease	Association of *PTPN22* allelic variants
RA	T1858: increased risk
	A788: decreased risk
	C-1123: increased risk in Asian populations
Systemic sclerosis	T1858: increased risk
	A788: none
SLE	T1858: increased risk
	A788: decreased risk
Primary Sjögren's syndrome	T1858: increased risk
Ankylosing spondylitis	T1858: none
Psoriatic arthritis	T1858: increased risk
Juvenile idiopathic arthritis	T1858: increased risk
	C-1123: increased risk
Giant cell arteritis	T1858: none
Takayasu's arteritis	T1858: none
Granulomatosis with polyangiitis (Wegener's granulomatosis)	T1858: increased risk
Microscopic polyangiitis	T1858: none
Churg-Strauss syndrome	T1858: none
Henoch-Schönlein purpura	T1858: none
Behcet's disease	T1858: decreased risk
Crohn's disease	T1858: decreased risk
	A788: none
Ulcerative colitis	T1858: none
	A788: decreased risk
Primary sclerosing cholangitis	T1858: none
Primary biliary cirrhosis	T1858: none
Celiac disease	T1858: none
T1D	T1858: increased risk
	C-1123: increased risk in Asian populations
Graves' disease	T1858: increased risk
Hashimoto's thyroiditis	T1858: increased risk
Primary Addison's disease	T1858: increased risk
Immune thrombocytopenic purpura	T1858: increased risk
Aplastic anemia	T1858: none
Alopecia areata	T1858: increased risk
Vitiligo	T1858: increased risk
Pemphigus vulgaris	T1858: none
Myasthenia gravis	T1858: increased risk
Acute anterior uveitis	T1858: none
Multiple sclerosis	T1858: none
Psoriasis	T1858: none

Carriers of the T1858 allele are significantly more likely to experience failure to respond to imatinib treatment for chronic myeloid leukemia than noncarriers, suggesting a role for LYP in regulating signaling downstream BCR-ABL in myeloid cells.

The T1858 allele frequency varies among populations, occurring most commonly in Scandinavians (greater than 10 % frequency), with lower frequency in Western Europeans (7–8 %), and even lower in Southern Europeans (2–3 %). The allele is extremely rare in African and Asian populations. In US and Australian Caucasians, it occurs at a frequency of 6–9 %, while it occurs at a frequency of 4–5 % in Hispanic populations. The reason for this geographical gradient is not yet clear, but evidence of positive selection at the *PTPN22* locus has been reported (Chung and Criswell 2007; Gregersen and Olsson 2009; Stanford et al. 2010).

To date, few studies have been conducted to investigate the role of *PTPN22* C1858T in infectious disease. The T1858 allele is protective against development of pulmonary tuberculosis (TB) and bacterial infections in immunocompromised transplant patients, but a risk factor for invasive pneumococcal infections, chronic mucocutaneous candidiasis and lepromatous and tuberculoid leprosy (Stanford et al. 2010). Thus far, the T1858 allele is shown to have no effect on hepatitis C infection outcome or susceptibility to *Trypanosoma cruzi* infection or brucellosis development (Burn et al. 2011). Further investigation of these diverging effects is likely, as with autoimmunity, to shed light on the functions of *PTPN22* in the immune system and in the pathogenesis of these disorders.

G788A/R263Q (rs33996649): This SNP lies within an exon encoding a segment of the catalytic domain, leading to an R263Q substitution that changes the conformation of the active site and reduces the enzymatic activity of the phosphatase. Carriers of this allele show reduced risk of developing several autoimmune diseases (Table 1), but increased risk of TB infection. This SNP segregates on different haplotypes than the C1858T SNP (Diaz-Gallo et al. 2011a; Orru et al. 2009; Stanford et al. 2010).

G-1123C (rs2488457): This SNP lies in the promoter region of *PTPN22* and has been shown to associate with several autoimmune diseases, particularly in Asian populations (Table 1). This SNP is in strict linkage disequilibrium with the C1858T SNP in Europeans and appears to have a minor effect in the presence of the C1858T SNP, but might be a significant risk factor in Asian populations (Burn et al. 2011; Stanford et al. 2010).

C1108A/H370N (rs72650671): This SNP is a rare variant encoding an H370N substitution in the interdomain region of the phosphatase and was recently found to increase risk of inflammatory bowel disease.

A10467572G (rs12730735): This SNP lies within intron 11 and was shown to associate with increased risk of Hashimoto's thyroiditis in a Korean population.

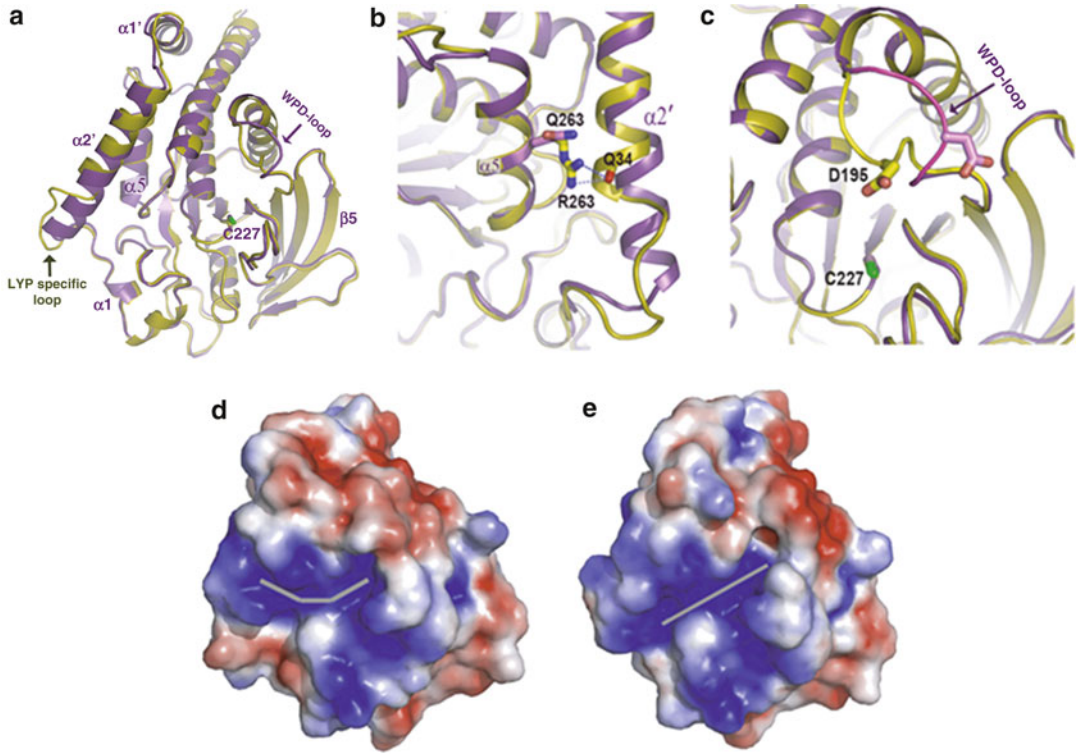
***PTPN22* Expression Levels and Autoimmune Disease**: Recent studies suggest that expression levels of *PTPN22* might also affect susceptibility to or prognosis of autoimmune disease. PTPN22 was shown to be expressed at higher levels in PBMC from patients with RA compared to controls. High *PTPN22* expression levels in $CD8^+$ T cells was shown to correlate with poor prognosis of antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis and SLE, suggesting PTPN22 might be a biomarker for prognosis for these or other autoimmune diseases.

Functional Genetics of the Disease-Associated PTPN22 Polymorphisms

The C1858T SNP encodes a substitution from arginine to tryptophan at amino acid 620 (R620W) of LYP, which strongly reduces the interaction between LYP and CSK. The functional effect of this variation is currently controversial. Some groups suggest that LYP-W620 is a gain-of-function form of the phosphatase. In support of this are reports that (1) T cells from T1D patients homozygous for the T1858 allele showed decreased TCR-induced Ca^{++} mobilization and IL-2 secretion (Rieck et al. 2007; Vang et al. 2005), (2) memory T cells from heterozygous T1858 carriers showed reduced TCR-induced Ca^{++} mobilization and IL-10 secretion (Rieck et al. 2007), and (3) LYP-W620 showed increased inhibition of TCR signaling and T cell activation compared to LYP-R620 in overexpression studies comparing the two variants (Fiorillo et al. 2010; Vang et al. 2005). A model to explain this gain-of-function phenotype of LYP-W620 at the molecular level is that the R620W polymorphism leads to increased enzymatic activity of LYP (see Fig. 3a, b). According to this model, impaired binding of LYP to CSK leads to decreased CSK-dependent recruitment of LCK to LYP and subsequent reduced phosphorylation of LYP on the inhibitory residue Y536. LYP-W620 is therefore a more potent inhibitor of TCR signaling, due, at least in part, to increased phosphatase activity (Fiorillo et al. 2010).

In contrast is the model suggesting that the W620 variant is a loss-of-function form of the phosphatase. In support of this model are two recent reports that (1) LYP-R620 was a more potent inhibitor of TCR stimulation compared to LYP-W620 when both variants were co-transfected with CSK in an overexpression study (Zikherman et al. 2009) and (2) T cells from mice carrying the W619 knock-in mutation of Pep (the homologous site to LYP-W620) and PBMC from homozygous carriers of the T1858 allele showed increased responsiveness to TCR engagement (Zhang et al. 2011). A model to explain the loss-of-function phenotype of LYP-W620 at the molecular level is that the R620W polymorphism leads to increased degradation of LYP (see Fig. 3c, d). According to this model, binding of LYP to calpain is enhanced in the presence of the W620 variation, leading to increased calpain- and proteasome-mediated cleavage of LYP-W620. LYP-W620 is therefore a less potent inhibitor of TCR signaling, due to decreased protein expression (Zhang et al. 2011).

It is evident that additional studies are necessary in order to explain the molecular effect of the R620W polymorphism on TCR signaling. No differences in transcription were observed between the two variants, suggesting that the molecular effect is at the protein level. At the systemic level, it is also not yet clear how



PTPN22, Fig. 4 Structural basis for the reduced activity of LYP-Q263. (**a**) Superimposed structures of the LYP-Q263 catalytic domain (Cat-Q263, shown in *purple*) on the LYP-R263 catalytic domain (Cat-R263, shown in *yellow*) show an open WPD loop and replacement of the LYP-specific loop with an extended helix in the mutant. The catalytic cysteine (C227) is shown in green. (**b**) The R263Q mutation in the LYP catalytic domain eliminates the contacts of R263 α5 helix to the α2' helix on the main-chain carbonyl oxygen of Q34. (**c**) Movement of the WPD loop to the open conformation in R263Q. (**d, e**) Surface electrostatics of the (**d**) Cat-R263 and (**e**) Cat-Q263 structures. The deep electropositive cleft stretches from the active site to the LYP-specific loop, which is marked by gray sticks. When the LYP-specific region adopts the helical conformation in Cat-Q263 and shifts away from residue 263, the cleft conformation changes, which may reduce the efficiency of substrate processing by the enzyme (This figure was reproduced from (Orrú et al. 2009) with permission from Oxford Journals)

the polymorphism abolishes immune tolerance to cause autoimmunity. Altered thresholds of TCR signaling could lead to (1) impaired deletion of autoreactive T cells during immune system development, (2) altered function of effector T cells, or (3) altered suppressive function of regulatory T cells, among others. Additionally, the role of LYP in B cells and in other immune cells also needs to be addressed. Carriers of the T1858 allele show decreased BCR signaling in circulating B cells and fewer memory B cells (Habib et al. 2012; Rieck et al. 2007). The B cell repertoire in these carriers seems to be skewed toward an increase in autoreactive T cells, suggesting that *PTPN22* might regulate B cell selection (Habib et al. 2012). The knock-in Pep-W619 mutation affects the DC phenotype in these mice, causing increased expansion of DCs and hyperresponsiveness of these cells to stimulation (Zhang et al. 2011). Further functional genetics of the role of the R620W polymorphism in both the adaptive and innate immune systems are necessary to clarify the pathogenesis of *PTPN22* in human disease.

Interestingly, the G788A polymorphism shows a disease association pattern quite distinct from the C1858T SNP (see Table 1). As shown in Fig. 4, this variation leads to an amino acid substitution in the catalytic domain of the phosphatase, changing the conformation of the LYP-specific loop of the active site (Orru et al. 2009). As this region participates in substrate binding, this variant shows reduced enzymatic activity and reduced inhibition of TCR signaling. To date, this SNP has not been shown to increase risk of any autoimmune disease but has been shown to confer protection against several. The A788 allele does, however, increase risk of TB, demonstrating an opposite effect to the T1858 allele (Diaz-Gallo et al. 2011a, b; Stanford et al. 2010).

Strategies to Inhibit LYP

An active area of research is the development of small molecule inhibitors of LYP. Due to the highly charged nature of the PTP active site, and because PTPs share a high degree of structural conservation near the active site, development of PTP inhibitors with cellular activity and selectivity over other PTPs is challenging. Several different strategies have been employed, yielding a few compounds with cellular activity. The first reported LYP inhibitor, called **I-C11**, is a salicylic acid-based compound developed by the Zhang group (Yu et al. 2007). **I-C11** was identified using a bidentate library screening approach, designed to identify ligands that bind to the active site of the enzyme and to an adjacent pocket. This binding mechanism was confirmed by structural analysis of **I-C11** co-crystallized with the catalytic domain of LYP. **I-C11** displays selectivity over a panel of PTPs and increases TCR signaling in a human cell line. Further optimization of this scaffold is ongoing and has successfully yielded additional compounds with activity in T cells (Barr 2010). Another approach used by the Barrios group has been to use Au(I)-phosphine complexes that bind covalently to cysteine residues in the catalytic domain. This strategy yielded a compound that shows increased TCR signaling in a human cell line and primary mouse T cells and shows high selectivity over the closely related PTP-PEST (Barr 2010). An in vivo study showed a Au(I)-phosphine complex effectively inhibited antigen-induced anaphylaxis in mice (Obiri et al. 2012). The first allosteric LYP inhibitor compound **4e** was identified from targeted screening of a small library of drug-like compounds to identify noncompetitive inhibitors. Structural studies indicate that this compound interacts with a hydrophobic patch located outside the active site of the enzyme. Treatment of a T cell line and primary T cells with compound **4e** and closely related analogs results in increased TCR signaling and T cell activation, and this compound shows some selectivity over the hematopoietic cell PTP, HePTP. All of these compounds inhibit LYP with an IC_{50} in the low micromolar range. In silico approaches led to the identification of compounds with sub-micromolar potency; however, selectivity or cellular activity of compounds identified in these studies has not yet been reported (Barr 2010).

Conclusion

The lymphoid phosphatase, LYP, is a hematopoietic non-receptor protein tyrosine phosphatase encoded by the *PTPN22* gene. LYP plays a key role as a gatekeeper of TCR signaling, and recent studies indicate that LYP is also involved in the signal transduction of multiple immune cell lineages and thus acts as a critical regulator of immune homeostasis. Functional variations in the *PTPN22* gene, in particular the R620W polymorphism, strongly affect susceptibility to multiple autoimmune diseases. This variant disrupts the interaction between LYP and CSK and results in increased risk of numerous autoimmune diseases and additional disorders and infections as well. While the mechanism of the pathogenesis of *PTPN22* in these disorders is not yet clear, it is evident that PTPN22 is a powerful gene that finely tunes the homeostasis of the immune system and immune tolerance.

Cross-References

- Alopecia Areata
- Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome)
- Genetics of Juvenile Idiopathic Arthritis
- Giant Cell Arteritis
- Immunology of Alopecia in Autoimmune Skin Disease
- Juvenile Diseases: SLE in Children
- Juvenile Idiopathic Arthritis: Pathogenesis, Presentation, and Treatment
- Lymphocytes in Atherosclerosis
- Macrophages, Oxidative Stress, and Atherosclerosis
- Microscopic Polyangiitis
- Primary Sclerosing Cholangitis: Clinical and Systemic Manifestations and Treatment
- Psoriasis
- Rheumatoid Arthritis, Clinical Features
- Rheumatoid Arthritis, Genetics
- Sjögren's Syndrome
- Spondyloarthritis: Ankylosing Spondylitis
- Spondyloarthritis: Psoriatic Arthritis
- Systemic Autoimmune Disease and Premature Atherosclerosis
- Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- Systemic Lupus Erythematosus, Genetics
- Systemic Lupus Erythematosus, Pathogenesis
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- Tregs in the Liver
- Vasculitis and the Kidney
- Vasculitis: Behçet
- Vasculitis: Granulomatosis with Polyangiitis (Wegener's)
- Vasculitis: Henoch-Schönlein Purpura

References

Barr AJ. Protein tyrosine phosphatases as drug targets: strategies and challenges of inhibitor development. Future Med Chem. 2010;2(10):1563–76.

Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet. 2004;75(2):330–7.

Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet. 2004;36(4):337–8.

Burn GL, Svensson L, Sanchez-Blanco C, Saini M, Cope AP. Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? FEBS Lett. 2011;585(23):3689–98.

Chung SA, Criswell LA. PTPN22: its role in SLE and autoimmunity. Autoimmunity. 2007;40(8):582–90.

Diaz-Gallo LM, Espino-Paisan L, Fransen K, Gomez-Garcia M, van Sommeren S, Cardena C, et al. Differential association of two PTPN22 coding variants with Crohn's disease and ulcerative colitis. Inflamm Bowel Dis. 2011a;17(11):2287–94.

Diaz-Gallo LM, Gourh P, Broen J, Simeon C, Fonollosa V, Ortego-Centeno N, et al. Analysis of the influence of PTPN22 gene polymorphisms in systemic sclerosis. Ann Rheum Dis. 2011b;70(3):454–62.

Fiorillo E, Orru V, Stanford SM, Liu Y, Salek M, Rapini N, et al. Autoimmune-associated PTPN22 R620W variation reduces phosphorylation of lymphoid phosphatase on an inhibitory tyrosine residue. J Biol Chem. 2010;285(34):26506–18.

Gregersen PK, Olsson LM. Recent advances in the genetics of autoimmune disease. Annu Rev Immunol. 2009;27:363–91.

Habib T, Funk A, Rieck M, Brahmandam A, Dai X, Panigrahi AK, et al. Altered B cell homeostasis is associated with type I diabetes and carriers of the PTPN22 allelic variant. J Immunol. 2012;188(1):487–96.

Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VE, et al. Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. Am J Hum Genet. 2004;75(3):504–7.

Obiri DD, Flink N, Maier JV, Neeb A, Maddalo D, Thiele W, et al. PEST-domain-enriched tyrosine phosphatase and glucocorticoids as regulators of anaphylaxis in mice. Allergy. 2012;67(2):175–82.

Orrú V, Tsai SJ, Rueda B, Fiorillo E, Stanford SM, Dasgupta J, et al. A loss-of-function variant of PTPN22 is associated with reduced risk of systemic lupus erythematosus. Hum Mol Genet. 2009;18(3):569–79.

Rieck M, Arechiga A, Onengut-Gumuscu S, Greenbaum C, Concannon P, Buckner JH. Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. J Immunol. 2007;179(7):4704–10.

Stanford SM, Mustelin TM, Bottini N. Lymphoid tyrosine phosphatase and autoimmunity: human genetics rediscovers tyrosine phosphatases. Semin Immunopathol. 2010;32(2):127–36.

Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P, et al. Autoimmune-associated lymphoid

tyrosine phosphatase is a gain-of-function variant. Nat Genet. 2005;37(12):1317–9.

Veillette A, Rhee I, Souza CM, Davidson D. PEST family phosphatases in immunity, autoimmunity, and autoinflammatory disorders. Immunol Rev. 2009; 228(1):312–24.

Yu X, Sun JP, He Y, Guo X, Liu S, Zhou B, et al. Structure, inhibitor, and regulatory mechanism of Lyp, a lymphoid-specific tyrosine phosphatase implicated in autoimmune diseases. Proc Natl Acad Sci USA. 2007;104(50):19767–72.

Zhang J, Zahir N, Jiang Q, Miliotis H, Heyraud S, Meng X, et al. The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness. Nat Genet. 2011;43(9): 902–7.

Zikherman J, Hermiston M, Steiner D, Hasegawa K, Chan A, Weiss A. PTPN22 deficiency cooperates with the CD45 E613R allele to break tolerance on a non-autoimmune background. J Immunol. 2009;182(7): 4093–106.

R

Raynaud's Phenomenon

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Synonyms

Raynaud's disease; Raynaud's syndrome

Definition

Raynaud's phenomenon is an exaggerated response to cold characterized by reversible, intermittent vasospasm of acral (extremity) arteries and arterioles.

Introduction

Primary Raynaud's phenomenon (PRP) or Raynaud's disease indicates the absence of an associated disease, drug, or occupational trigger, whereas secondary Raynaud's phenomenon (SRP) refers to the vasospastic condition, which may accompany an underlying disorder such as a connective tissue disease. The classic description of RP is the occurrence of a triphasic color change to the digits starting with pallor (white) as a result of decreased blood flow, followed by cyanosis (blue) due to deoxygenation of static venous blood and finally rubor (red) from reperfusion hyperemia; however, many patients do not recall or report three distinct color changes. While this disorder was first described by Maurice Raynaud in 1862, more than 150 years later, many questions remain regarding the exact pathophysiology and ideal treatment.

Prevalence and Risk Factors

Population studies of RP estimate disease prevalence between 3 % and 9 % in the general population (Fraenkel et al. 1999; Harada et al. 1991). Gender, hormones, genetics, environment, body mass index, and occupational exposures may all contribute to disparity in prevalence across studies. Overall, there is a well-established female predominance in RP. Risk factors for RP may vary between genders. For example, emotional stress may be more predictive of Raynaud's development among women, whereas occupational exposure and smoking may be more prominent risk factors among men (Fraenkel 2002).

Estrogen, in particular, has been linked to development of RP and may explain the high rate of onset of PRP during puberty. Additionally, postmenopausal females receiving unopposed estrogen therapy had an odds ratio of developing RP of 2.5 compared with postmenopausal females not given HRT. Patients that use estrogen and progesterone in combination do not have an increased risk of RP (Fraenkel et al. 1998).

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The existence of genetic risk factors is supported by the increased prevalence of RP in patients who have family members with RP and differences between ethnic groups living in the same environment (Valter and Maricq 1998). Maricq et al. compared the prevalence of RP in 5 climates. There was a considerable difference in the rate of occurrence between warm and cold climates, suggesting that subclinical cold injury may be the predisposing factor for increased RP in the colder climates (Maricq et al. 1997). Lower BMI (or weight loss) may increase the risk of RP, especially among women (Roquelaure et al. 2012).

Disease Associations

Systemic sclerosis (SSc) is the connective tissue disease most commonly associated with SRP, with greater than 90 % of SSc patients developing RP during their disease course. RP is seen to a lesser extent in other autoimmune diseases including systemic lupus erythematosus, dermatomyositis and polymyositis, rheumatoid arthritis, undifferentiated connective tissue disease, and Sjogren's syndrome (Khan 1999). Other associations with RP include hypothyroidism, use of certain medications, and atherosclerotic disease (Table 1). Repetitive trauma (usually occupational exposure) to the superficial palmar branch of the ulnar artery causes hypothenar hammer syndrome, which may represent around 1 % of all Raynaud's presentations (Marie et al. 2007). This may be reversible with elimination of the trauma and conservative management.

Raynaud's Phenomenon, Table 1 Conditions associated with Raynaud's phenomenon

Rheumatologic diseases	
	Systemic Sclerosis
	Mixed Connective tissue disease
	Polymyositis/Dermatomyositis
	Undifferentiated Connective tissue disease
	Overlap syndromes
	Systemic vasculitis
	Systemic lupus erythematosus
	Rheumatoid Arthritis
	Sjogren's syndrome
Endocrine	
	Hypothyroidism
Hematologic/ oncologic	
	Cryoglobulinemia
	Cryofibrinogenemia
	Anti-Phospholipid syndrome
	Polycythemia
	Paraneoplastic syndromes
Obstructive vasculopathy	
	Atherosclerosis
	Embolic disease
	Thromboangiitis obliterans
	Thoracic outlet syndrome
Occupational exposure	
	Vibratory injury (hypothenar hammer syndrome, etc.)
	Frostbite
	Polyvinyl chloride
Drugs/Chemicals	
	Beta-blockers
	Cocaine
	Nicotine
	Caffeine
	Bleomycin and Vincristine
	Ergotamine
	Estrogen

Diagnosis of Primary and Secondary Raynaud's phenomenon

Raynaud's phenomenon is typically diagnosed by a clinical description of cold sensitivity in the digits with the observation of associated color changes. Confirmatory tests are not routinely performed in clinical practice. However, in the research realm, measurement of changes in digital systolic blood pressure before and after cooling the extremities in 15 °C water is a diagnostic tool. A drop in pressure greater than 20–25 mmHg may be indicative of RP (Galarraga et al. 2008). Other techniques for diagnosis include digital thermography and laser Doppler flowmetry.

It may be difficult to distinguish between PRP and SRP at the initial patient evaluation since RP may be the presenting symptom of an evolving connective tissue disease. A thorough history and physical examination (with particular attention to the skin, joints and muscles) are valuable in establishing a diagnosis of SRP. Several key features may be utilized for stratifying the risk of future development of a connective tissue disease. PRP tends to present in the second and third decades and has a milder disease course. Older age, rapid disease onset, and digital pits and ulcers are features of SRP. The occurrence of digital ulceration and tissue necrosis, rarely seen in PRP, should prompt a thorough evaluation for an underlying cause.

Autoantibody testing is also helpful for risk stratification at RP presentation. In a study of vascular studies and autoantibody testing, Landry et al. found that among patients with vasospasm who had negative RF and ANA tests, only 2 % progressed to connective tissue disease over 10 years while nearly 30 % of those with positive tests and arterial obstruction were ultimately diagnosed with a connective tissue disease (Landry et al. 1996). Similarly, Ziegler et al. found ANA was helpful for predicting progression to CTD whereas baseline ESR and CRP were not prognostic indicators (Ziegler et al. 2003).

In addition to baseline laboratory studies, nailfold capillaroscopy is useful in predicting which subset of Raynaud's patients will develop connective tissue diseases. In a 5-year prospective study, the finding of "scleroderma type" capillary changes – thickened and enlarged loops with capillary dropout – on initial evaluation was highly predictive for development of a connective tissue disease (systemic sclerosis, dermatomyositis, overlap syndrome, and mixed connective tissue disease) (Pavlov-Dolijanovic et al. 2012).

Pathophysiology

Although the pathophysiologic underpinnings of RP have not been fully elucidated, much is known about the mechanisms that contribute to this disorder. Because SRP, unlike PRP, is associated with such a diverse group of conditions and factors (see above), it is reasonable to infer that its causation involves different mechanisms, alone or in combination. Yet, there are certain distinct abnormalities which play a crucial role in the overt clinical manifestation of RP. These abnormalities may be broadly broken down into those involving the blood vessel wall, neural vascular control, and intravascular factors.

Abnormalities of the Blood Vessel

In PRP, the vascular defect is thought to be primarily functional in nature (Herrick 2005). Thus, while the blood vessels in these individuals are structurally intact, they demonstrate an exaggerated response to cold exposure and emotion. Cold triggers vasoconstriction through the effect of norepinephrine on the vascular smooth muscle. Therefore, the RP may be mediated by smooth muscle contraction as a result of increased sympathetic neural activity.

The vascular endothelium plays a crucial role both in vasoregulation and in controlling the growth of surrounding connective tissue (Limaye et al. 2007). When activated, endothelial cells produce a variety of cytokines which may contribute to structural vascular changes such as those seen in scleroderma and associated SRP. Among the more important of these cytokines is the vasoconstrictor, endothelin-1. Its overexpression is now well recognized as a key element in the pathogenesis of the microvascular abnormalities noted in scleroderma. Importantly, increased levels of endothelin-1 are also associated with SRP, especially when accompanied by digital ulcers. In pulmonary hypertension, which may complicate scleroderma, there is also increased expression of endothelin-1 in vascular endothelial cells and in the circulation (Giaid et al. 1993). This is the rationale for using bosentan, an endothelin-1 receptor antagonist, to treat both of these complications and its potential use for recalcitrant Raynaud's phenomenon (see below).

Neurovascular Dysregulation

The sympathetic nervous system plays a central role in the regulation of the peripheral circulation.

Vasoconstriction is mediated in part through the release of certain neuropeptides such as substance P and calcitonin gene–related peptide. Increased reactivity of alpha-2 adrenergic receptors in response to norepinephrine also contributes to the cold-induced vasospasm of PRP and SRP (Cooke and Marshall 2005). It is likely that an imbalance favoring vasoconstrictor over vasodilatory nerve activity results in reduced peripheral blood flow and what is recognized clinically as RP. Since calcitonin gene–related peptide released from sensory fibers is among the most important mediators of vasodilation, a reduction in number of these nerve fibers or damage to them would predispose to the development of a RP. Vibration-induced RP is well recognized in occupational medicine community. In this setting, neurovascular dysregulation appears to play a central role in the etiology of the vasospasm. Here, persistent exposure to vibratory tools causes direct injury to and depletion of the vasoregulatory nerves of the hands (Stoyneva et al. 2003).

Intravascular Abnormalities

A number of blood cellular elements and circulating factors may have important roles in the pathogenesis of RP associated with scleroderma. Increased levels of the vasoconstrictors thromboxane and serotonin attest to platelet activation in this condition (Herrick 2005). Furthermore, some scleroderma patients have been shown to have defective fibrinolysis. Fibrin deposition in smaller vessels can produce both compromised blood flow and microvascular occlusions (Ames et al. 1997).

Cryoglobulinemia and cryofibrinogenemia may be associated with RP, perhaps due to increased blood viscosity. Reduced red blood cell deformity also may contribute to impaired flow through peripheral vessels.

It is important to keep in mind that both vascular and neural factors play important roles in the pathogenesis of PRP and SRP. Since the control of peripheral blood flow is a complex interplay between central and local factors, perturbations in one or more of these regulatory mechanisms can trigger vasospasm.

Treatment

General Measures

The initial approach to managing RP is one of life-style modification. The emphasis should be on reducing cold exposure and sudden temperature change. When cold exposure is anticipated, the use of chemical or battery-powered warmers for gloves and socks may be helpful. In the workplace, patients should wear protective clothing with an emphasis on warming the extremities and maintaining core body temperature. For some individuals, a change in occupation may be necessary. It is also best to avoid drugs which may cause exacerbations of RP such as beta-blockers, amphetamines, and caffeine. Smoking cessation and avoidance of secondhand smoke is also advantageous. The use of behavioral therapy in RP is controversial. A meta-analysis of 5 studies utilizing biofeedback failed to demonstrate improvement in RP severity, frequency, or duration (Malenfant et al. 2009). In the case of SRP, treatment of the underlying condition is important as well.

Drug Therapy

Since RP appears to reflect an imbalance between vasodilation and vasoconstriction, pharmacologic treatment may be thought of broadly as the use of agents that promote vasodilation or those that inhibit vasoconstriction.

Vasodilators

- Calcium channel blockers (CCB): The most commonly utilized vasodilators are non-cardioselective calcium channel blockers. Inhibition of voltage-gated calcium channels leads to relaxation of vascular smooth muscle, causing vasodilation. A meta-analysis of PRP in 2005 showed a significant reduction in frequency (2.8 to 5.0 attacks less per week) and severity of attacks (33 % reduction) in the CCB treatment group versus placebo (Thompson and Pope 2005). The most commonly used CCB is nifedipine which is started at low doses and titrated upward as needed and tolerated. Other CCBs such as felodipine and amlodipine may also be of benefit. The use of

CCBs in patients with relative hypotension can be problematic and requires careful monitoring. Side effects including headache and lower extremity edema may limit tolerance of these medications. Unfortunately, the effect of CCBs tends to diminish over time.

- Topical Nitrates: Compounds such as glycerol trinitrate and 2 % nitroglycerin gel have been shown to improve RP and digital ulcer severity when applied topically (Franks 1982). They are typically utilized to improve blood flow to one digit, particularly when ulceration has occurred. Topical nitrate therapy for RP has been limited by the side effects of hypotension, headache, dizziness, and local skin irritation. A recent trial evaluating a new formulation of topical nitrate with faster absorption for local vasodilation was aimed at improving the side-effect profile of topical nitrates and is the largest placebo-controlled trial to date. While this study did show improvement in Raynaud's condition score, there were no statistically significant changes in Raynaud's frequency or severity (Chung et al. 2009).
- Phosphodiesterase type 5 inhibitors (sildenafil, vardenafil, and tadalifil): These agents have been used in RP in an attempt to improve peripheral circulation. They act by enhancing the effect of nitric oxide in promoting vascular smooth muscle relaxation. However, these drugs have had mixed results in mitigating recurrent attacks. One study of patients with severe RP reported a decrease in attacks and disease severity, and a tendency toward ulcer healing (Herrick et al. 2011). It has been suggested that this class of drugs is most effective when added to calcium channel blocker therapy.
- Prostaglandins: Both parenteral and oral prostaglandins have been utilized to decrease Raynaud's severity; in addition to vasodilation, they inhibit platelet aggregation and vascular proliferation. Intravenous formulations of prostaglandins analogues have been utilized in the setting of severe RP with digital ulcerations and digital necrosis. One placebo-controlled trial using a five-day course of 6-h intravenous infusions of iloprost showed a 39.1 % decrease in weekly number of Raynaud's attacks versus a 22 % decrease with placebo and an improved Raynaud's severity score (Wigley et al. 1994). Due to the difficulty of administration of the parental formulation, multiple cyclic schedules have been utilized for sustained effect. However, these have not been FDA approved for use in Raynaud's in the USA (Milio et al. 2006). Oral prostaglandins have not shown consistent benefit. A six-week trial of oral iloprost for management of Raynaud's in systemic sclerosis failed to show a reduction in frequency or duration of attacks compared to placebo (Wigley et al. 1998).

Inhibitors of vasoconstriction

- Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs): ACE inhibitors and ARBs act as indirect vasodilators by inhibiting the effect of angiotensin II which is a powerful vasoconstrictor. The studies of ACE inhibitors in RP have generally been of short duration and have had mixed results and have not been shown to have a significant effect versus placebo (Gliddon et al. 2007). The ARB losartan when compared to the CCB nifedipine has shown a significant reduction in frequency and severity of Raynaud's attacks (Dziadzio et al. 1999).
- Endothelin receptor antagonists: These agents may be utilized as a second- or third-line treatment. Since endothelin-1 is a potent vasoconstrictor and is implicated in vascular remodeling, fibrosis, and inflammation, it is a potential target particularly in SRP with digital ulcerations. Treatment with the endothelin receptor antagonist bosentan was associated with a 48 % reduction in the mean number of new ulcers compared with placebo after 16 weeks of treatment (Korn et al. 2004). Another study utilizing bosentan over a 24-week course, compared to placebo, showed a 30 % reduction in occurrence of new digital ulcers; the reduction of new ulcers was more pronounced among patients with multiple ulcers (Matucci-Cerinic et al. 2011).

Other drugs

- Selective serotonin reuptake inhibitors (SSRIs) are another class of drugs that produce indirect vasodilation by blocking uptake of serotonin, a known vasoconstrictor. Thus, SSRIs provide one option for Raynaud's treatment in patients with relative hypotension. In a study utilizing fluoxetine 20 mg daily (versus a comparator group of nifedipine 40 mg daily), there was a statistically significant decrease in attack severity and frequency after 6 weeks of treatment (Coleiro 2001). Subgroup analysis showed the greatest effect in females with PRP.
- Anticoagulation is not routinely utilized in RP or for digital ulcerations but may be used during the acute phase of significant digital gangrene.

Sympathectomy

The disruption of the proximal or digital sympathetic nervous system can decrease the vasospastic response to cold. Sympathectomy may be either chemical or surgical. One tool for local chemical sympathectomy is injection of botulinum toxin A to block vasoconstriction of the digital arteries (Neumeister 2010). While case reports have shown significant improvement in healing of ulcers and a lessening of Raynaud's severity, there is a lack of randomized controlled data to support its use. In the setting of severe digital pain and vasospasm, a local digital or wrist block using lidocaine may be helpful.

Surgical sympathectomy may be performed proximally in the cervical region or at the digital level. Digital sympathectomy is usually preferred due to its less invasive nature and less severe side-effect profile. While a reduction in amputation for digital ischemia is reported in SRP, digital ulcerations following surgical digital sympathectomy may still occur (Wasserman and Brahn 2010).

Conclusion

Raynaud's phenomenon is a cold or emotion-induced vasospastic process affecting the digits. It is clear that the environment, hormones, and genetics play important roles in its pathogenesis. While RP is usually an isolated finding, it is important to keep in mind its important associations with connective tissue disease especially in patients who present with symptoms of obstructive arteriopathy, such as digital infarcts and ulceration. Despite major advances in understanding of the pathophysiology of RP, more effective treatments are needed for this condition.

Cross-References

- ▶ Scleroderma: Genetics
- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Ames PR, Lupoli S, Alves J, et al. The coagulation/fibrinolytic balance in systemic sclerosis: evidence for a haematological stress syndrome. Br J Rheumatol. 1997;36:1045–50.

Chung L, Shapiro L, Fiorentino D, et al. MQX-503, a novel formulation of nitroglycerin, improves the severity of Raynaud's phenomenon. Arthritis Rheum. 2009;60(3):870–7.

Coleiro B, Marshall SE, Denton CP, et al. Treatment of Raynaud's phenomenon with selective serotonin reuptake inhibitor fluoxetine. Rheumatology. 2001; 40:1038–43.

Cooke J, Marshall J. Mechanisms of Raynaud's disease. Vasc Med. 2005;10:293–307.

Dziadzio M, Denton CP, Smith R, et al. Losartan therapy for Raynaud's phenomenon and scleroderma. Arthritis Rheum. 1999;42(12):2646–55.

Fraenkel L. Raynaud's phenomenon: epidemiology and risk factors. Curr Rheumatol Rep. 2002;4:123–8.

Fraenkel L, Zhang Y, Chaisson C, et al. The association of estrogen replacement therapy and the Raynaud phenomenon in postmenopausal women. Ann Intern Med. 1998;129(3):208–11.

Fraenkel L, Zhang Y, Chaisson C, et al. Different factors influencing the expression of Raynaud's phenomenon in men and women. Arthritis Rheum. 1999;42(2): 306–10.

Franks AG. Topical glyceryl trinitrate as adjunctive treatment in Raynaud's disease. Lancet. 1982; 1(8263):76–7.

Galarraga B, Al-Allaf AW, Belch JJF. Raynaud's phenomenon. Chapter 135. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Rheumatology. 4th ed. New York: Mosby; 2008. p. 1417–22.
Giaid A, Yanagisawa M, Langleben D, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. N Engl J Med. 1993;328:1732–9.
Gliddon AE, Dore CJ, Black CM, et al. Prevention of vascular damage in scleroderma and autoimmune Raynaud's phenomenon. Arthritis Rheum. 2007;56(11):3837–46.
Harada N, Ueda A, Takegata S. Prevalence of Raynaud's phenomenon in Japanese males and females. J Clin Epidemiol. 1991;44(7):649–55.
Herrick AL. The pathogenesis of Raynaud's phenomenon. Rheumatology. 2005;44:587–96.
Herrick AL, van den Hoogan F, Gabrielli A, et al. Modified-release sildenafil reduces Raynaud's phenomenon attack frequency in limited cutaneous systemic sclerosis. Arthritis Rheum. 2011;63(3):775–82.
Khan F. Vascular abnormalities in Raynaud's. Scott Med J. 1999;44(1):4–6.
Korn JH, Mayes M, Matucci-Cerinic M, et al. Digital ulcers in systemic sclerosis: prevention by treatment with Bosentan, an oral endothelin receptor antagonist. Arthritis Rheum. 2004;50(12):3985–93.
Landry GJ, Edwards JM, McLafferty RB, et al. Long-term outcome of Raynaud's syndrome in a prospectively analyzed patient cohort. J Vasc Surg. 1996;23(1):76–85.
Limaye Y, Vadas M. The vascular endothelium: structure and function. In: Fitridge R, Thompson M, editors. Mechanisms of vascular disease. Cambridge: Cambridge University Press; 2007. p. 1–10.
Malenfant D, Catton M, Pope JE. The efficacy of complementary and alternative medicine in the treatment of Raynaud's phenomenon: a literature review and meta-analysis. Rheumatology. 2009;48:791–5.
Maricq HR, Carpentier PH, Weintich MC, et al. Geographic variation in the prevalence of Raynaud's phenomenon: a 5 region comparison. J Rheumatol. 1997;24(5):879–89.
Marie I, Herve F, Primard E, et al. Long-term follow-up of hypothenar hammer syndrome: a series of 47 patients. Medicine (Baltimore). 2007;86(6):334–43.
Matucci-Cerinic M, Denton CP, Furst DE, et al. Bosentan treatment of digital ulcers related to systemic sclerosis: results from the RAPIDS-2 randomized, double-blind, placebo controlled trial. Ann Rheum Dis. 2011;70:32–8.
Milio G, Corrado E, Genova C, et al. Iloprost treatment in patients with Raynaud's phenomenon secondary to systemic sclerosis and the quality of life: a new therapeutic protocol. Rheumatology. 2006;45:999–1004.
Neumeister MW. Botulinum toxin type A in the treatment of Raynaud's phenomenon. J Hand Surg Am. 2010;35(12):2085–92.
Pavlov-Dolijanovic S, Damjanov NS, Stojanovic RM, et al. Scleroderma pattern of nailfold capillary changes as predictive value for the development of a connective tissue disease: a follow-up study of 3,029 patients with primary Raynaud's phenomenon. Rheumatol Int. 2012;32(10):3039–45.
Roquelaure Y, Ha C, Le Manac'h AP, et al. Risk factors for Raynaud's phenomenon in the workforce. Arthritis Care Res. 2012;64(6):898–914.
Stoyneva Z, Lyapina M, Tzvetkov D, Vodenicharov E. Current pathophysiological views on vibration-induced Raynaud's phenomenon. Cardiovasc Res. 2003;57:615–24.
Thompson AE, Pope JE. Calcium channel blockers for primary Raynaud's phenomenon: a meta-analysis. Rheumatology. 2005;44:145–50.
Valter I, Maricq HR. Prevalence of Raynaud's phenomenon in 2 ethnic groups in the general population of Estonia. J Rheumatol. 1998;25(4):697–702.
Wasserman A, Brahn E. Systemic sclerosis: bilateral improvement of Raynaud's phenomenon with unilateral digital symptathectomy. Semin Arthritis Rheumix. 2010;40(2):137–46.
Wigley FM, Wise RA, Seibold JR, et al. Intravenous Iloprost infusion in patients with Raynaud phenomenon secondary to systemic sclerosis. Ann Intern Med. 1994;120:199–206.
Wigley FM, Korn JH, Csuka ME, et al. Oral iloprost treatment in patients with Raynaud's phenomenon secondary to systemic sclerosis: a multicenter, placebo-controlled, double blind study. Arthritis Rheum. 1998;41(4):670–7.
Ziegler S, Brunner M, Eigenbauer E, et al. Long-term outcome of primary Raynaud's phenomenon and its conversion to connective tissue disease: a 12 year retrospective patient analysis. Scand J Rheumatol. 2003;32:343–7.

Regulatory B Cells

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Synonyms

B10 B cells; Bregs; Killer B cells; Suppressor B cells; T2-like Bregs

Definition

Regulatory B cells are $CD19^+$ lymphocytes that can inhibit inflammation in vivo or suppress the activation of immune cells in vitro or in vivo.

Introduction

The primary function of B cells is to participate in adaptive immune responses, acting as the precursor cells for immunoglobulin-producing plasma cells, presenting antigen to T cells and iNKT cells, and producing Th1 and Th2 cytokines that support the activation and differentiation of other lymphocytes. In certain circumstances, a particular subset, or subsets, of B cells, or a temporally/experimentally induced functional group of B cells, can play a role in controlling inflammation both in vivo and in vitro. These B cells have been named regulatory B cells (Bregs) (DiLillo et al. 2010; Mauri and Bosma 2012).

B cells with a regulatory function have been identified in a number of mouse models, primarily of autoimmunity but also infection, transplantation, and cancer, and have been ascribed a number of partially overlapping phenotypes (Mauri and Bosma 2012; Kalampokis et al. 2013). The factors involved in their generation and suppressive function vary according to the model used. In addition, regulatory B cells have been reported in human peripheral blood (Blair et al. 2010). At present, there is no equivalent of the regulatory T cell transcription factor Foxp3 that defines regulatory $CD4^+$ T cells (Tregs), to definitively discriminate Bregs from other B cells in either mice or humans, and the question of whether Bregs are a developmentally discrete subset or a functional classification that overlaps with previously defined B cell subsets is yet to be answered.

Historical Background

A suppressive capacity was first ascribed to B cells in the 1970s when it was found that adoptive transfer of B cell-depleted splenocytes was unable to prevent delayed-type hypersensitivity (DTH) skin reactions in guinea pigs in the way that adoptive transfer of B cell-replete splenocytes was able to. It was initially hypothesized that suppressor B cells might produce suppressive antibody that blocked the activity of cells responsible for DTH reactions (Katz et al. 1974). This hypothesis was later rejected when it was found that suppression of DTH could occur in the absence of detectable antibody production. The next reports of suppressive B cells were published in the mid-1980s when it was found that B cells were associated with the generation of antigen-specific T suppressor cells. However, the study of regulatory B cells did not begin in earnest until the late 1990s following the work of Charles Janeway who unexpectedly found that B cell-deficient mice were unable to recover from experimental autoimmune encephalomyelitis (EAE, a mouse model of multiple sclerosis) (Mann et al. 2012). The study of B cell-suppressive capacity then broadened to take in mouse models of rheumatoid arthritis, systemic lupus erythematosus (SLE), diabetes, inflammatory bowel diseases, and also studies of infection. The use of the term "regulatory B cell" to describe B cells that are able to suppress inflammation in vivo was first introduced in the early 2000s (Mizoguchi and Bhan 2006).

Subsets and Markers of Murine Regulatory B Cells

There is currently no consensus surface (cluster differentiation, CD) marker phenotype for murine regulatory B cells, and this phenotype has varied depending on the models studied. In the field of autoimmunity, two phenotypes that allow for the purification of functional regulatory B cells have achieved some prominence. $CD19^+$ $CD21^+$ $CD23^+$ $CD24^+$ IgM^+ IgD^+ B cells, termed T2-like Bregs, express surface markers consistent with belonging to an immature transitional-2 stage of development and have been reported to suppress T cell activation in vitro and to inhibit arthritis and lupus-like

disease in vivo upon adoptive transfer (Mauri and Bosma 2012). Similarly, adoptive transfers of CD1d^{hi} CD5^{+} B cells, termed B10 B cells for their ability to produce the immunoregulatory cytokine interleukin-10 (IL-10), have been reported to control DTH responses, EAE, and lupus-like disease (DiLillo et al. 2010). Although different CD markers are used to define T2-like Bregs and B10 B cells, there is some overlap in their phenotypes: both populations express CD19, CD21, CD24, and CD1d, and both can be isolated from naïve splenic B cells. It is possible that these phenotypes reflect the same population or developmentally related regulatory B cell subsets.

Other models of autoimmunity have produced partial phenotypes for regulatory B cells. A spontaneous colitis model resulting from the knockout of the α subunit of the T cell receptor (TCRα$^{-/-}$ model of colitis) suggested that the expression of CD1d, IgM, CD21, and CD23 may be associated with regulatory B cells. However, B cells purified based on this phenotype were not directly tested in suppression assays or by adoptive transfer. In models of rheumatoid arthritis and multiple sclerosis, marginal zone B cells and pre-plasma cell-like B cells have also been ascribed suppressive function (Mizoguchi and Bhan 2006).

Recently the study of transplantation has provided another possible marker for murine regulatory B cells (Ding et al. 2011). Treatment of mouse pancreatic islet transplant recipients with an antibody to T cell Ig domain and mucin domain protein 1 (TIM-1) induces the expansion of regulatory TIM-1^{+} B cells and tolerance to the allograft. The adoptive transfer of TIM-1^{+} B cells prolonged allograft survival. In contrast to the phenotypes defining T2-like Bregs and B10 Bregs, which appear to describe discrete B cell populations, TIM-1 is expressed by a wide range of developmentally distinct B cell subsets. This discrepancy highlights the fact that it is as yet unclear whether regulatory B cells are a developmentally discrete subset of B cells or whether many B cell subsets may contain potential regulatory B cells given the right immunological environment (Ding et al. 2011).

Human Regulatory B Cell Subsets

The study of regulatory B cells in humans is in its infancy. However, there is growing evidence that B cells with a CD19^{+} CD24^{hi} CD38^{hi} phenotype may comprise regulatory B cells. As with murine T2-like regulatory B cells, this phenotype is reminiscent of B cells at an immature transitional stage of development. The initial description of human regulatory B cells came from a study of multiple sclerosis patients who had concurrent helminth infections (Correale et al. 2008). Helminth infection correlated with lower numbers of relapses to disease with the induction of regulatory T cells, and it restored the ability of naïve CD27^{-}IgD^{+} B cells to produce IL-10 in response to CD40 stimulation. Purified B cells from helminth-infected patients could suppress the proliferation of, and IFN-γ production by, myelin-specific T cells in vitro. Shortly after this work, it was reported that CD19^{+} CD24^{hi} CD38^{hi} B cells purified from human healthy peripheral blood suppressed T cell activation in vitro. Again, this was a primarily CD40-driven IL-10-mediated suppression. Moreover CD19^{+} CD24^{hi} CD38^{hi} B cells shared the expression of CD1d and IgD with the B cells identified in multiple sclerosis patients and were also predominantly CD27^{-}(Blair et al. 2010).

CD19^{+} CD24^{hi} CD38^{hi} Bregs have been confirmed to be important clinically. In SLE, CD19^{+} CD24^{hi} CD38^{hi} Bregs are defective and lack the ability to of healthy Bregs to suppress T cell cytokine production in vitro (Blair et al. 2010). In rheumatoid arthritis, although these cells retain their suppressive capacity, they are reduced in number in the peripheral blood of patients with active disease (Flores-Borja et al. 2013). Interestingly, following treatment with the B cell-depleting anti-CD20 antibody Rituximab, remission from SLE correlates with the persistence of CD19^{+} CD24^{hi} CD38^{hi} B cells following B cell repopulation (Anolik 2011). In the field of transplantation, higher numbers of CD19^{+} CD24^{hi} CD38^{hi} B cells were detected in the peripheral blood of immunosuppressant-free tolerant recipients of a kidney transplant as compared to patients who required immunosuppression to

maintain graft function (Chong and Sciammas 2011). In contrast to their potentially beneficial role in autoimmunity and transplantation, $CD24^{hi}$ $CD38^{hi}$ Bregs may contribute to HIV-associated immune dysfunction of T cells: the frequency of PD-$L1^{+}$ $CD24^{hi}$ $CD38^{hi}$ B cells correlates with markers of HIV disease progression and may promote T cell dysfunction via the production of IL-10 (Siewe et al. 2013).

Other phenotypes for human regulatory B cells have been suggested. Human peripheral blood $CD24^{hi}$ $CD27^{+}$ B cells are described to produce the highest level of IL-10 in vitro following lipopolysaccharide (LPS) stimulation. It was hypothesized that these cells may be human equivalents of murine B10 Bregs. Functionally, these cells were able to inhibit TNF-α production by monocytes in vitro; however, this suppression was IL-10 independent. Clinically, $CD24^{hi}$ $CD27^{+}$ B cell numbers have not been reported to be decreased in patients with systemic or organ-specific autoimmune diseases. Moreover, IL-10 production in response to anti-CD40 and LPS or CpG stimulation was significantly increased in patient $CD24^{hi}$ $CD27^{+}$ B cells as compared to healthy controls (Iwata et al. 2011; Kalampokis et al. 2013).

Mechanisms of Suppression of Regulatory B Cells

At a cellular level, regulatory B cells can have multiple suppressive effects: they can (1) inhibit T cell cytokine production and proliferation, (2) convert Th1- to Th2-type immune responses, (3) inhibit the activation of antigen-presenting cells, (4) induce the generation of other regulatory cell subsets, or (5) cause the apoptosis of activated lymphocytes (Mauri and Bosma 2012).

The primary mechanism for the function of regulatory B cells has consistently been reported to be the production of the immunoregulatory cytokine IL-10. Studies of T2-like Bregs, B10 Bregs, and TIM-1^{+} Bregs have all found that if the regulatory B cell subsets are isolated from IL-10-deficient mice, or if IL-10 is blocked using monoclonal antibodies, then these cells are no longer able to inhibit autoimmune or alloimmune responses in vivo. It is hypothesized that IL-10 produced by B cells acts directly on T cells, or antigen-presenting cells, to suppress their activation. Similarly, in humans, the production of IL-10 appears to be a central feature of proposed regulatory B cell subsets, and IL-10 blockade can reverse their ability to suppress T cell cytokine production in vitro (DiLillo et al. 2010; Ding et al. 2011; Mauri and Bosma 2012).

IL-10 production is not the whole story, and different models of inflammation have identified different effector mechanisms for regulatory B cells. For instance, IL-10 production was initially found to be crucial for regulatory B cells to control inflammation in the $TCR\alpha^{-/-}$ model of colitis. It was subsequently found, however, that suppression also required IL-12-producing B cells, which may have been the cells that were directly mediating the inhibition of Th2 colitis (Sugimoto et al. 2007). In the NOD mouse model of diabetes, regulatory B cells have been shown to work via the production of transforming growth factor β (TGFβ), which induces apoptosis or anergy of $CD8^{+}$ diabetogenic T cells (Mauri and Bosma 2012). Similarly, schistosome infection can induce FASL expression on B cells, which can then go on to suppress $CD4^{+}$ T cell responses by FASL-FAS-induced cell death. These B cells were termed "killer B cells," although they may represent a type of regulatory B cell. Human B cells have also been reported to express FASL following mitogenic stimulation, although whether this has a role in suppression is as yet unclear (Klinker and Lundy 2012).

Antigen specificity is another feature of regulatory B cell function in mouse models. T2-like Bregs and B10 Bregs have both been shown to work in an antigen-specific manner in mouse models of arthritis and DTH, respectively. However, the clearest demonstration of regulatory B cell antigen specificity comes from the study of TIM-1^{+} regulatory B cells in transplantation. Here, TIM-1^{+} regulatory B cells isolated from mice that had been tolerized to pancreatic islet transplants from donors expressing the $H\text{-}2^{K}$ MHC haplotype were unable to prolong the

survival of pancreatic islet transplants from mice expressing the H-2^d MHC haplotype. In humans a requirement for antigen specificity has not yet been proven (Ding et al. 2011).

Regulatory B cells may mediate their suppressive effect through the induction of other regulatory cell subsets. In mouse models of arthritis, SLE, and multiple sclerosis, regulatory B cells have been shown to induce type 1 regulatory (Tr1) or $Foxp3^+$ regulatory T cells. In humans, $CD19^+$ $CD24^{hi}$ $CD38^{hi}$ regulatory B cells are able to convert $CD4^+$ $CD25^-$effector T cells into regulatory T cells (Mauri and Bosma 2012). In addition, it has been proposed that regulatory B cells may interact with invariant natural killer T cells (iNKT cells). iNKT cells are a subset of T cells that recognize lipid antigens presented by CD1d, a molecule expressed by the majority of murine and human regulatory B cell subsets. Although the recruitment of iNKT cells by regulatory B cells has not yet been formally proven, it has been demonstrated that CD1d expression is required for regulatory B cells to suppress colitis in mice, as B cells from CD1d knockout mice are unable to prevent intestinal inflammation (Mizoguchi and Bhan 2006).

A number of other cell surface receptors have been associated with regulatory B cell function. In particular, regulatory B cells that lack the expression of CD40 or CD86 are unable to control murine colitis. Similarly, in humans, blocking CD86 with monoclonal antibodies prevents $CD19^+$ $CD24^{hi}$ $CD38^{hi}$ regulatory B cells from suppressing T cell cytokine production in vitro. Both CD40 and CD86 expression by regulatory B cells may be important for their ability to interact with activated T cells that express their respective ligands, CD154 and CD28.

The Generation of Regulatory B Cells

While regulatory B cells can be isolated from naïve mice or directly from the peripheral blood of healthy individuals, it has been a consistent feature across most studies of regulatory B cells that their function can be enhanced, or their numbers expanded, by the presence of inflammation. T2-like regulatory B cells are empowered by the inflammation associated with collagen-induced arthritis; accordingly, inhibition of arthritis can be achieved by adoptively transferring half the number of these cells isolated from arthritic mice as compared to naïve mice (Mauri and Bosma 2012). Similarly, adoptive transfers of $TIM\text{-}1^+$ regulatory B cells can only prolong survival of pancreatic islet transplants if they are isolated from mice that have previously received islet allografts and been subject to transplant-associated inflammation (Ding et al. 2011).

Inflammation may induce regulatory B cell function through a number of pathways. First of all, signals from activated inflammatory T cells have been associated with the generation of regulatory B cells. For instance, activated $CD4^+$ T cells express CD154, the ligand for CD40, and ligation of CD40 using anti-CD40 antibodies can induce regulatory B cell IL-10 production and rescue suppressive function of defective Bregs. Both T2-like Bregs and B10 Bregs achieve their full potential for IL-10 production when stimulated in vitro via anti-CD40 (DiLillo et al. 2010; Mauri and Bosma 2012). In addition, T2-like Bregs isolated from lupus-prone MRL/*lpr* mice require in vitro stimulation with anti-CD40 to become regulatory as they are defective directly ex vivo. Cytokine production by activated T cells is equally important. Activated $CD4^+$ T cells produce IL-21 that enhances IL-10 production by regulatory B cells. Moreover, in vitro cultures of B cells with IL-21 and cells expressing CD154 and B cell-activating factor (BAFF) induce a potent $CD5^+$ regulatory B cell population that suppresses EAE development and can reverse established EAE (Yoshizaki et al. 2012).

In addition to signals from activated T cells, cognate interactions between B cells and antigen may be required for regulatory B cell development. It has been observed that mice deficient in CD19, a molecule that participates in B cell receptor (BCR) signal transduction, develop exacerbated EAE compared to wild-type mice, suggesting that BCR signals may be important in generating regulatory B cells. Similarly,

transgenic mice whose B cells all have BCRs specific for hen egg lysozyme, a protein not found in mice, have lower frequencies of IL-10^+ B cells. This suggests that B cells need to meet their cognate antigen for development of a regulatory IL-10 response (Mauri and Bosma 2012).

Regulatory B cell interaction with the innate immune system is another potential enhancer of regulatory B cell function. B cells express a number of receptors that are specific for bacterial fragments or innate danger signals, and several groups have found a requirement for signaling through these innate pattern recognition receptors for regulatory B cell function (Lampropoulou et al. 2010). For instance, B10 Bregs require stimulation with LPS, a ligand for Toll-like receptor (TLR) 4, to achieve full IL-10 production in vitro. Similarly, in humans and mice, it is well documented that CpG, an oligonucleotide ligand for TLR-9, can induce B cell IL-10 production. Treatment of mice with apoptotic cells can also generate regulatory B cells that are able to control collagen-induced arthritis. It has not been formally proven how this effect is mediated, but it could involve stimulation of B cells through TLRs as the recipient B cells pick up pieces of apoptotic material. Interestingly, one of the receptors for phosphatidyl serine, a molecule exposed on the membranes of apoptotic cells or vesicles, is TIM-1, which as described above is a marker of regulatory B cells in transplantation. Thus, apoptotic material may directly activate regulatory B cells through TIM-1. TIM-1 expression by regulatory B cells may in addition facilitate their interaction with innate immune cells such as monocytes or dendritic cells, both of which express one of its ligands, TIM-4 (Miyanishi et al. 2007).

Regulatory B cells can also be enhanced in vitro using vitamins or synthetic molecules. For instance, the active form of vitamin D, calcitriol, enhances B cell IL-10 production in vitro as compared to anti-CD40 and IL-4 stimulation alone. Functional regulatory B cells can be generated from mouse splenocytes cultured with a fusion protein GIFT15 constructed by coupling IL-15 to the cytokine granulocyte macrophage colony-stimulating factor (GM-CSF). GIFT15 generates IL-10-producing regulatory B cells that can suppress EAE development upon adoptive transfer (Deng and Galipeau 2012).

Conclusion

Regulatory B cells have been reported in a wide range of settings, mainly in the field of experimental autoimmunity. They exist in both rodents and humans and have been ascribed several phenotypes and modes of action, with IL-10 production being suggested as a primary function. Although they have been shown to be efficient suppressors of inflammation upon adoptive transfer, their role in the natural course of inflammation resolution is yet to be properly studied. Regulatory B cells appear to be generated by the process of inflammation itself and are much more potent when isolated from an inflammatory rather than naïve microenvironment. The ability to enhance regulatory B cell activity in vitro, and the findings that they can suppress inflammation following adoptive transfers in the mouse, has led some to suggest that, like regulatory T cells, regulatory B cells could one day be used for cellular therapy in humans.

Cross-References

- ▶ B cell Tolerance
- ▶ BCR Signaling
- ▶ CD40
- ▶ Liver Transplantation Tolerance in Animal Models for Encyclopedia of Medical Immunology
- ▶ Rheumatoid Arthritis, Treatment
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Systemic Lupus Erythematosus, Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver

References

Anolik J. Immunologic reconstitution after rituximab in systemic lupus erythematosus: why should we care? J Rheumatol. 2011;38:587–9.

Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, Mauri C. CD19(+) CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. Immunity. 2010;32:129–40.

Chong AS, Sciammas R. Matchmaking the B-cell signature of tolerance to regulatory B cells. Am J Transplant. 2011;11:2555–60.

Correale J, Farez M, Razzitte G. Helminth infections associated with multiple sclerosis induce regulatory B cells. Ann Neurol. 2008;64:187–99.

Deng J, Galipeau J. Reprogramming of B cells into regulatory cells with engineered fusokines. Infect Disord Drug Targets. 2012;12:248–54.

Dilillo DJ, Matsushita T, Tedder TF. B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer. Ann N Y Acad Sci. 2010;1183:38–57.

Ding Q, Yeung M, Camirand G, Zeng Q, Akiba H, Yagita H, Chalasani G, Sayegh MH, Najafian N, Rothstein DM. Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. J Clin Invest. 2011;121:3645–56.

Flores-Borja F, Bosma A, NG D, Reddy V, Isenberg DA, Ehrenstein MR, Mauri C. CD19 + CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation. Sci Transl Med. 2013;5:173ra23.

Iwata Y, Matsushita T, Horikawa M, Dilillo DJ, Yanaba K, Venturi GM, Szabolcs PM, Bernstein SH, Magro CM, Williams AD, Hall RP, ST Clair EW, Tedder TF. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood. 2011;117:530–41.

Kalampokis I, Yoshizaki A, Tedder TF. IL-10-producing regulatory B cells (B10 cells) in autoimmune disease. Arthritis Res Ther. 2013;15:S1.

Katz SI, Parker D, Turk JL. B-cell suppression of delayed hypersensitivity reactions. Nature. 1974;251:550–1.

Klinker MW, Lundy SK. Multiple mechanisms of immune suppression by B lymphocytes. Mol Med. 2012;18:123–37.

Lampropoulou V, Calderon-Gomez E, Roch T, Neves P, Shen P, Stervbo U, Boudinot P, Anderton SM, Fillatreau S. Suppressive functions of activated B cells in autoimmune diseases reveal the dual roles of Toll-like receptors in immunity. Immunol Rev. 2010;233:146–61.

Mann MK, Ray A, Basu S, Karp CL, Dittel BN. Pathogenic and regulatory roles for B cells in experimental autoimmune encephalomyelitis. Autoimmunity. 2012;45:388–99.

Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol. 2012;30:221–41.

Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S. Identification of Tim4 as a phosphatidylserine receptor. Nature. 2007;450:435–9.

Mizoguchi A, Bhan AK. A case for regulatory B cells. J Immunol. 2006;176:705–10.

Siewe B, Stapleton JT, Martinson J, Keshavarzian A, Kazmi N, Demarais PM, Landay A, French AL. Regulatory B cell frequency correlates with markers of HIV disease progression and attenuates anti-HIV CD8+ T cell function in vitro. J Leukoc Biol. 2013;93:811–8.

Sugimoto K, Ogawa A, Shimomura Y, Nagahama K, Mizoguchi A, Bhan AK. Inducible IL-12-producing B cells regulate Th2-mediated intestinal inflammation. Gastroenterology. 2007;133:124–36.

Yoshizaki A, Miyagaki T, Dilillo DJ, Matsushita T, Horikawa M, Kountikov EI, Spolski R, Poe JC, Leonard WJ, Tedder TF. Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. Nature. 2012;491:264–8.

Regulatory T Cell

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Synonyms

Treg

Definition

Tregs: Regulatory T cells – Regulatory T cells, or suppressor T cells, are a distinct T cell population that downregulates the immune response. They play a central role in maintaining self tolerance and the prevention of autoimmunity. More recently, they have been shown to modulate many immune responses in infectious disease and cancer settings.

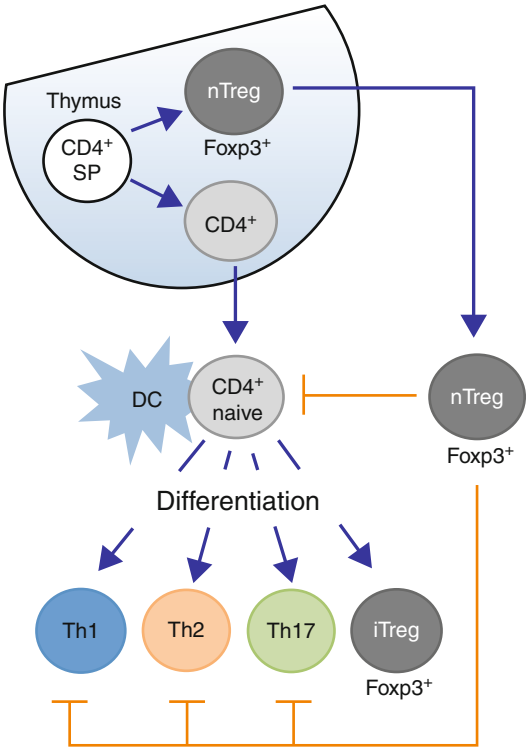
Historical Background

The immune system is tightly regulated to facilitate responses to infection while at the same time avoiding collateral damage of noninfected cells and avoiding immune activation to self-antigens that can result in autoimmunity. Cytokines made by effector T cells play a major role in activating and inhibiting immune functions, in essence making all immune cells regulatory in nature. However, since the 1970s, the idea of a specialized T cell whose primary function is to suppress immunity has been hotly debated and widely studied (Sakaguchi et al. 2008; Shevach 2000). The first breakthrough in defining Tregs was the identification of regulatory T cell suppressive function within a population of T cells expressing CD4 and CD25 (the interleukin-2 (IL-2) receptor α chain) (Sakaguchi et al. 1995). Depletion of CD25-expressing cells in the mouse led to autoimmunity that could be completely prevented by the transfer of $CD4^+CD25^+$ T cells from healthy donors (Sakaguchi et al. 1995). A second milestone came with the identification of the transcription factor Foxp3 (forkhead box P3) expressed only by $CD4^+CD25^+$ T cells in the steady state and which acts as a master regulator of Treg development and function (Josefowicz and Rudensky 2009). Naturally occurring mutations in the X chromosome-encoded *Foxp3* gene eliminate Treg generation in the thymus and lead to a lethal multiorgan autoimmune/inflammatory disease in mice (Scurfy) and human (IPEX) (Bennett et al. 2001; Brunkow et al. 2001). Thus, self-tolerance or homeostatic control of the immune system is dependent on regulation by thymically derived $CD4^+CD25^+Foxp3^+$ T cells that hold in check autoreactive T cells in the periphery. More recently, it has become evident that Tregs are an integral component of all adaptive immune responses being recruited and activated alongside effector T cells. The balance between Tregs and effectors has been shown to impact the quality or magnitude of immunity to many immune challenges including pathogens, tumors, transplantation, and vaccination.

Regulatory T Cell Subsets

Regulatory T cells can be divided into two main groups: thymically derived Tregs, which are generated in the thymus and seed the periphery as a functionally mature subset of T cells with regulatory function (natural Tregs or nTregs), and extrathymically derived Tregs, which can be induced from naïve T cells in the periphery following cognate antigen recognition (induced Tregs or iTregs) (Fig. 1). Both Treg populations express the Foxp3 gene that controls their development and function. Independent of Foxp3, a number of other T cells have been shown to be suppressive largely through the production of immune-suppressive cytokines such as IL-10 and TGF-β. These cells include suppressive Tr1 and Th3 $CD4^+$ cells, $CD8^+$ T cells, CD4-CD8- T cells and γδ T cells (Shevach 2006). These additional suppressive T cell subsets do not appear to play a critical role in self-tolerance but have been shown to impact the magnitude of the adaptive immune response in inflammatory settings. Of note, regulatory B cells expressing IL-10 have also been described, and their role in self-tolerance and immune regulation is currently of great interest.

Thymically Derived nTregs: The importance of the thymus in generating regulatory T cells has been appreciated for some 30 years. Early studies in the mouse demonstrated that thymectomy at day 3 of life resulted in organ-specific autoimmunity, while thymectomy on day 7 of life did not result in autoimmunity. Autoimmunity could be prevented in the day 3-thymectomized mice by the transfer of lymphocytes from adult thymus or spleen. Therefore, after 3 days of life, the thymus generates a population of T cells capable of controlling self-reactivity. The identification of Foxp3 as a Treg lineage-specific transcription factor fueled studies on the early development of regulatory T cells in the thymus (Josefowicz and Rudensky 2009). Similar to conventional $CD4^+$ and $CD8^+$ αβ T cell development in the thymus, Treg thymic development is a stepwise process that is dependent on signals through the TCR, CD28, and cytokine receptors.



Regulatory T Cell, Fig. 1 *Regulatory T cell development*. Regulatory T cells require the expression of the lineage specific transcription factor Foxp3 that controls Treg development, maintenance and function. Tregs can develop in the thymus (natural, nTregs) or be induced from conventional naive CD4 T cells in lymphoid tissues (induced, iTregs). (1) Bone marrow-derived single positive CD4+ T cells in the thymus are selected into the conventional CD4+ T cell lineage (T-helper-cells, Th) or into the CD4+Foxp3 + Regulatory T cell lineage. Signals for Treg development in the thymus include a limited antigen-specific niche, CD28 costimulation and interleukin-2 (IL-2). (2) In the peripheral lymphoid tissues, conventional naive CD4+ T cells are activated on encounter cognate ligand presented in the context of MHC Class II by dendritic cells (DC). The cytokine millieu at the time of initial activation drives the differentiation of naive T cells into effector T cells with specialized functions (Th1, for clearence of intracellular pathogens such as bacteria and viruses; Th2, for the clearance of large extracelluar parasites such as helminths; and Th17 for the clearance of some extracellular bacteria and fungi). In addition to effector T cells, naive T cells can differentiate into regulatory T cells (iTreg) by encounter with DCs in the presence of IL-2, TGFβ and retinoic acid. The gut-associated lymphoid tissues is a major site of iTreg generation. Tregs can inhibit the initial activation of naive T cells in the lymph nodes and their subsequent effector function in infected/inflamed tissues using a variety of mechanisms.

TCR specificity plays a critical role in determining Treg development in the thymus but not in a straightforward manner in which TCRs can be classified as being destined to induce Tregs or to induce conventional T cells. Rather, the ability of TCRs to facilitate Treg development appears to be controlled by limited antigen-specific niches, with the TCR clonal frequency needing to be below 1 % for efficient Treg selection (Lio and Hsieh 2011). Such a mechanism, although not fully understood, is likely to result in Treg TCRs recognizing rare tissue-specific antigens rather than ubiquitously expressed self-antigens and lead to a highly diverse TCR repertoire important for broad control of autoreactivity in the periphery. Along with TCR engagement, thymic development of Tregs requires signals from CD28 and the downstream activation of NF-κB, specifically c-Rel. Another critical signal for Treg differentiation is interleukin-2 (IL-2) mediated through the IL-2R and the signal transducer and activator of transcription 5 (STAT5). This combination of signals is thought to induce and/or stabilize Foxp3 gene expression during Treg differentiation but may also help to drive the expansion or survival of differentiated Tregs in the thymus. In the periphery, IL-2 produced by conventional T cells plays a key role in the maintenance of Foxp3 expression and in the activation, expansion, and survival of nTregs.

Peripherally Induced iTregs: The original studies of the Foxp3 gene demonstrated that ectopic expression of Foxp3 in nonregulatory T cells in vitro conferred regulatory functions, highlighting the fundamental role that Foxp3 plays in orchestrating the regulatory program. In vivo, naïve conventional T cells can be induced to express Foxp3 and gain regulatory function under distinct activation conditions. iTreg differentiation appears to be favored by interactions with dendritic cells (DCs) in the absence of inflammation leading to suboptimal TCR signaling or by activation in tolerogenic microenvironments such as the gut (Bilate and Lafaille 2012). In vitro, differentiation of naïve T cells into iTregs is dependent on antigen-specific activation by DCs in the presence of IL-2 and TGF-β and is

inhibited by IL-6. In contrast, TGF-β in combination with IL-6 promotes Th17 differentiation that is reciprocally inhibited by IL-2. The central role of TGF-β in driving both regulatory and effector populations suggests that iTregs and Th17 development are closely linked and that cofactors provided by the inflammatory milieu will dictate immune tolerance or activation. In vivo, the gastrointestinal tract represents a major site for iTreg development mediated through T cell activation by a particular retinoic acid producing $CD103^+$ DC subset in the gut-associated lymphoid tissue (GALT) (Siddiqui and Powrie 2008). Retinoic acid promotes iTreg differentiation by augmenting Foxp3 induction and inhibiting IL-6-dependent induction of Th17s. The induction of iTregs in the gut is thought to provide the mechanistic basis for tolerance/nonresponsiveness to orally administered antigens, known as oral tolerance. iTregs can also be generated in chronic inflammatory conditions such as allergic asthma, autoimmunity, and chronic infection. Induction of iTregs in inflammatory settings is still dependent on TGF-β but the antigen-presenting cell (APC) types and cytokine milieu that differentially drive effector and regulatory T cell differentiation in inflammation are not well defined. Kinetically, iTregs develop later than effector cells in an inflammatory immune response and are thought to limit the tissue damage caused by effector T cells.

Foxp3 and Treg Plasticity: In the past 10 years, the field of T cell differentiation has become heavily focused on stability of effector T cell subsets and the possibility of functional plasticity (Nakayamada et al. 2012). Th1 and Th2 cells were once thought to be stable "terminally differentiated" lineages by virtue of lineage-specific transcription factor-guided chromatin remodeling that opened up cytokine loci of the one program and repressed the cytokine loci of the reciprocal program. Technical advances in our ability to measure chromatin modifications have facilitated a detailed analysis of epigenetic marks in differentiated effector cells and have revealed that the lineage-specific transcription factors for the opposing lineage are poised for transcriptional activation. Indeed, otherwise stable, Th2 cells were shown to express Tbet and IFN-γ when activated in the context of interferons induced by viral infection. Therefore, Th effector populations can be functionally reprogrammed in particular inflammatory environments. For Tregs, the Foxp3 locus is heavily demethylated in nTregs indicating stability of the lineage (Miyara et al. 2009). In contrast, the Foxp3 locus of TGF-β-induced iTregs is more methylated implicating functional instability (Baron et al. 2007). Recent cell fate mapping studies using fluorescently tagged $Foxp3^+$ T cells have shown in certain circumstances that Foxp3 expression can be lost and that this loss coincides with the gain of effector function (Zhou et al. 2009), a study that remains controversial. The possibility of instability in the Treg lineage however and the ability of Tregs to gain effector function will need to be closely monitored as efforts to use Treg cell therapy for controlling inflammation and autoimmunity continue to be developed.

Of note, unlike mice, human conventional $CD4^+$ T cells express Foxp3 upon activation but do not acquire regulatory T cell phenotype or function. In this setting, Foxp3 is expressed at lower levels than nTregs and is transient and therefore may not be present in the cell long enough to make fundamental changes to effector function. Therefore, in humans, additional markers are needed to accurately distinguish between $Foxp3^+$ recently activated effector T cells and $Foxp3^+$ Tregs. The level of expression of the interleukin-7 receptor α, CD127, inversely correlates with Foxp3 expression and is widely used to distinguish Tregs from effector T cells, Tregs being $CD4^+CD25^{hi}Foxp3^{hi}CD127^{lo}$ (Miyara and Sakaguchi 2011).

Function

Regulatory T cells have been shown to regulate many immune cell types including $CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, macrophages, mast cells, and dendritic cells. This broad spectrum of suppression is facilitated by the

Regulatory T Cell, Table 1

nTreg molecules	Regulatory mechanism
Cell surface	
CTLA-4	– Competes for co-stimulatory CD80/86 ligand binding on DC; reduces naive T cell activation – Removes CD80/86 from DC cell surface by endocytosis; reduces naïve T cell activation – Binding to CD80/86 induces IDO expression in DC, suppresses T cell activation by local depletion of tryptophan, induces a regulatory phenotype in the DC
CD25	Competes for the growth factor IL-2; limits naive T cell expansion/survival
LFA-1/ neuropilin	High Treg cell surface expression enhances Treg-DC adhesion; may physically limit naïve T cell interactions with DC
CD39/CD73	Ectoenzymes that degrade ATP and generate adenosine; limits availability of ATP needed for T cell activation signals, adenosine-mediated inhibition of cytokine transcription, role in metabolic inhibition
Surface TGF-β LAP	Broad immunoregulation; inhibits leukocyte activation and function; modulates DC function
LAG3	An MHC class II-binding CD4 homolog, implicated as a negative regulator; function unknown but LAG3-deficient Tregs have reduced suppressive function
Secreted	
IL-10	Broad immunoregulation; blocks effector cytokines such as IFN-γ at barrier surfaces; inhibits macrophage activation, suppresses Th1 responses
TGF-β	Broad immunoregulation; inhibits CD8 cytolytic function; supports DC generation of iTregs
IL-35	Heterodimeric regulatory cytokine composed of IL-12 p35 and Ebi3; inhibits T cell activation and cytokine production; can induce new IL-35-producing regulatory T cells
Perforin/ granzymes	Cytotoxic; can induce apoptosis in a variety of immune cells
cAMP	High intracellular levels in Tregs, delivered to T cell targets via gap junctions; inhibits T cell activation and cytokine production, role in metabolic inhibition

expression of a wide variety of adhesion molecules and chemokine receptors that enable Treg trafficking to lymphoid tissues, to inflamed tissues at infection and autoimmune sites, and to tumors (Campbell and Koch 2011). Tregs deficient in CD103 (αE integrin) failed to home to inflamed tissues and were functionally compromised. Thus, Tregs are localized for control of early events in immune activation in the lymph node (proliferation/expansion) and subsequent effector functions (cytokines, cytolytic) in inflamed/infected tissues. Multiple mechanisms for Treg suppressive function have been identified that fall into three main categories: cell-cell contact, secretion of soluble factors (cytokines), and competition for growth factors (Sojka et al. 2008; Tang and Bluestone 2008) (Table 1). The relative importance of each suppressive mechanism will likely depend on the type of target cell, its functional stage, and the activation milieu at the time of regulation. Recent advances in the ability to delete genes specifically in the Treg lineage will help to elucidate the role of specific regulatory components.

Cell-Cell Contact Dependent: The classic in vitro test of Treg function is a suppression assay in which conventional $CD4^+$ T cells are activated by anti-CD3 antibodies and APCs (T-depleted splenocytes) in the presence or absence of Tregs. In the presence of Tregs, $CD4^+$ T cells fail to sustain gene expression for IL-2 and do not proliferate. Suppression is lost if the Tregs and target $CD4^+$ T cells are physically separated using a transwell system. Therefore, cell-cell contact appears to be required for regulatory action, or, more precisely, the Treg and target T cells and/or antigen-presenting cells need to be in close proximity. A variety of different Treg mechanisms work through cell-cell contact, either Treg-APC or Treg-T conventional.

Cell contact between the Treg and the antigen-presenting cells, namely, DCs for naïve T cell activation, limits the stimulatory capacity of DC in a number of ways and hence limits T cell

activation. T cell activation is dependent on co-stimulation through CD28 on the T cell binding to CD80 and CD86 on the APC. CTLA-4 is a negative regulator of T cell activation that has a higher affinity for CD80/CD86 than CD28 and can outcompete CD28 for ligand binding and hence attenuate activation signals. Tregs constitutively express CTLA-4 on their surface and therefore can block conventional T cell access to DC CD80/CD86 co-stimulation and prevent activation of naïve T cells. It has also been reported that Tregs can limit co-stimulation by the physical removal of CD80/86 from the surface of the APC by CTLA-4-mediated trans-endocytosis. Tregs also express higher levels of adhesion molecules, such as LFA-1, and may dominantly interact with the DC at the expense of naïve T cells. Indeed, in vivo imaging studies have shown that Tregs inhibit stable contacts between the conventional T cells and DC. Tregs may also change the stimulatory properties of DC by inducing functional changes in the APC. Engagement of Treg CTLA-4 with CD80/86 on the DC initiates DC upregulation of indoleamine 2,3-dioxygenase (IDO). IDO is a tryptophan-catabolizing enzyme that suppresses T cell activation by local depletion of tryptophan and, independent of its catabolic functions, induces a regulatory phenotype in the DC.

Tregs can also directly interact with the T cells themselves to block activation. Tregs express high intracellular levels of cyclic AMP (cAMP) and have been shown to be able to directly deliver cAMP to the T cells via gap junctions. cAMP is a potent inhibitor of many cytokine genes in the T cells. In some settings, such as in the tumor microenvironment, the expression of the cytotoxic molecules granzyme and perforin by Tregs appears to limit antitumor immunity by inducing T cell and DC apoptosis. Although not directly cell-cell contact, Tregs can alter local concentrations of ATP through their surface expression of the ectoenzymes CD39 and CD73 that catalyze the generation of adenosine from ATP and ADP. Extracellular ATP is important for activated T cell signaling, and therefore, Treg-mediated degradation of ATP limits resources for efficient T cell activation. In addition, adenosine binds to A2A receptors on effector T cells and inhibits cytokine transcription via induction of cAMP.

Secretion of Soluble Factors: Tregs secrete a number of immunosuppressive cytokines including TGF-β, IL-10, and a newly described suppressive cytokine IL-35. Although not found to be required for the in vitro assays of Treg suppression of T cell proliferation, in vivo these cytokines clearly play a role in the regulatory function of Tregs. TGF-β is a key regulator of immune homeostasis and is made by many cell types including Tregs. Disruption of TGF-β signaling in T cells leads to a fatal lymphoproliferative disease similar to that seen with Foxp3 deficiency. TGF-β responsiveness by $CD8^+$ effector T cells was found to be key to Treg-mediated inhibition of cytolytic function. In addition to inhibiting immunity, TGF-β is also important for inducing and maintaining Foxp3 expression and thus positively regulates Tregs, both the generation of iTregs and the maintenance of nTregs. IL-10 is also produced by a range of immune cell types and can inhibit macrophage activation, induce tolerogenic DCs, and block Th17 generation. Treg-specific depletion of IL-10 revealed a nonredundant role for Treg IL-10 at barrier surfaces with the mice developing amplified lung, skin, and intestinal pathologies. Indeed, IL-10-deficient Tregs retained the ability to inhibit early T cell activation and proliferation in the lymph node but were unable to control IFN-γ production in the dermis. These studies highlight that although Tregs have a large arsenal of immunosuppressive tools, particular suppressive mechanisms will be critical in distinct locations or at specific time points in a developing immune response.

Competition: Regulatory T cells constitutively express a number of cell surface molecules that provide a competitive advantage for resources over conventional T cells where the molecules are not constitutively expressed but need to be induced following activation. CTLA-4 expression has been discussed earlier with respect to the ability of Tregs to outcompete naïve T cells for CD80/CD86 on DCs. IL-2 is fundamental to Treg survival and function but is not made by the Tregs themselves. Expression of the high-affinity

IL-2R, experimentally defined by the IL-2Rα chain CD25, facilitates the rapid utilization of exogenous IL-2 made largely by naïve T cells after activation. Tregs can therefore deprive conventional T cells of IL-2 and limit their proliferation and survival. Such a mechanism may be particularly important during early activation events in the lymph node where IL-2 is essential for antigen-specific T cell expansion.

Tregs in Disease

Mutations in the Foxp3 gene that result in loss of function lead to an early and fatal multiorgan autoimmune/inflammatory disease in mice and man (Bennett et al. 2001; Brunkow et al. 2001), demonstrating the importance of Treg control of self-tolerance and immune homeostasis. Therefore, there has been great interest in determining if a defect in Treg number or function can explain the loss of self-tolerance that defines autoimmune diseases. Acute deletion of Tregs in animal models can precipitate autoimmune disease, and the provision of additional Tregs can prevent disease. In humans, Treg functional defects have been described for type 1 diabetes (T1D), rheumatoid arthritis (RA), multiple sclerosis, and systemic lupus erythematosus (Buckner 2010). In RA, functional defects in Tregs may be caused by the inflammatory milieu of the disease (particularly TNF-α) and can be reversed by removing Tregs from the microenvironment or neutralizing the inflammatory cytokines. In T1D, in addition to Treg functional defects, the effector T cells from diabetic subjects appear to be more resistant to suppression adding another mechanistic layer to the immune dysregulation seen in autoimmunity. There is much excitement about the possibility of Treg therapy in autoimmune disease with efforts to define conditions in which to expand and/or induce antigen-specific Tregs for specific autoimmune diseases. However, successful Treg therapy will need to confront the functional plasticity of Treg populations and the nature of the inflammatory environment in which the Tregs will be required to function.

Tregs are also key to the control of immune responses to infection. By limiting the effector T cell response, Tregs prevent local immunopathology resulting from excessive cytokine production. Clearly, there is a fine balance between overcoming suppression sufficiently to mount a robust antimicrobial immune response and controlling collateral damage in the tissues. This balance is regulated at many levels with inflammatory signals that both inhibit Treg activity but also promote Treg specialization and enhance Treg function (Campbell and Koch 2011). Layered on top of this hematopoietic self-control is the ability of many pathogens to promote Treg recruitment and/or activation in infected tissues to repress pathogen-specific effector responses and promote/prolong microbial residency in the host (Maizels and Smith 2011). High levels of inflammatory cytokines such as TNF-α, IFN-γ and IL-6 have been shown to dampen nTreg activity and inhibit the generation of iTregs. In RA, anti-TNF-α treatment correlates with enhanced Treg activity. However, Tregs are also capable of responding to these same effector cytokines in a positive way to adapt to the inflammatory environment and provide better regulation. A series of recent studies have shown that, under effector T cell-polarizing conditions, Tregs acquire signaling and transcriptional components normally expressed by the effector T cells themselves (Campbell and Koch 2011). In a Th1-inducing environment, Tregs express Tbet which facilitates their recruitment to sites of Th1 inflammation. Similarly, in Th2 inflammation, Tregs appear to require expression of the Th2-associated transcription factor interferon regulatory factor 4 (IRF4) to curtail aberrant IgG1 and IgE production. For control of Th17 responses, Treg need STAT 3, a transcription factor downstream of the IL-6R. In all these cases, deletion of these molecules in Tregs compromised their ability to adapt to the environmental conditions and led to an inflammation-specific reduction in their regulatory capacity. Therefore, there is a complex relationship between inflammation and Treg activity, and the efficacy of Treg function in

a given inflammatory setting will likely be highly context dependent.

One unexpected negative consequence of Treg immune control has come from the study of tumor-infiltrating lymphocytes. Regulatory T cells expressing CCR4 are readily recruited to tumors by CCL22-producing tumor cells and tumor-infiltrating macrophages. The tumor can also promote iTreg generation through high TGF-β production and the induction of tolerogenic DCs. Tregs are highly activated in the tumor and can functionally dominate the tumor microenvironment preventing T cell activation. Indeed, an increased ratio of Tregs to $CD8^+$ T cells in the tumor correlates with poor prognosis in patients with breast, gastric, and ovarian cancers. Depletion of Tregs has been shown to lead to rapid T cell-mediated destruction of tumors and has prompted efforts to design therapies that block Treg activity in human tumor settings.

Summary

$Foxp3^+CD25^+CD4^+$ regulatory T cells are key modulators of immunity for immune tolerance and homeostasis and in response to infectious challenge. The Treg lineage is defined by the expression of the transcription factor Foxp3 that acts as a "master regulator" of Treg development and function. Tregs can limit the stimulatory capacity of DCs, outcompete naïve T cells for resources, inhibit effector cytokine production, and even kill immune cells. Mechanistically, Tregs express an array of immunosuppressive tools either on their cell surface such as CTLA-4 and CD25 which outcompete naïve T cells for activation signals or by secreted factors such as the immunosuppressive cytokines IL-10 and TGF-β. A loss of Treg function leads to a systemic autoimmune/inflammatory disease, highlighting their critical role in immune homeostasis and the prevention of autoimmunity. Tregs also play an integral role in immune responses to infection and other immune challenges such as allergens, vaccination, tumors, and transplantation. The balance between Treg and effector T cell responses is tightly regulated, and a number of therapeutic strategies are being developed to exploit this control mechanism to boost Treg activity in autoimmune and inflammatory diseases and inhibit Tregs for better tumor clearance.

Cross-References

- ▶ B7 and CD28 Families
- ▶ CTLA-4
- ▶ Regulatory B Cells
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Baron U, Floess S, Wieczorek G, Baumann K, Grutzkau A, Dong J, Thiel A, Boeld TJ, Hoffmann P, Edinger M, Turbachova I, Hamann A, Olek S, Huehn J. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. Eur J Immunol. 2007;37(9):2378–89.

Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001;27(1):20–1.

Bilate AM, Lafaille JJ. Induced CD4(+)Foxp3(+) regulatory T cells in immune tolerance. Annu Rev Immunol. 2012;30:733–58.

Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001;27(1):68–73.

Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol. 2010;10(12):849–59.

Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. Nat Rev Immunol. 2011;11(2):119–30.

Josefowicz SZ, Rudensky A. Control of regulatory T cell lineage commitment and maintenance. Immunity. 2009;30(5):616–25.

Lio CW, Hsieh CS. Becoming self-aware: the thymic education of regulatory T cells. Curr Opin Immunol. 2011;23(2):213–9.

Maizels RM, Smith KA. Regulatory T cells in infection. Adv Immunol. 2011;112:73–136.

Miyara M, Sakaguchi S. Human FoxP3(+)CD4(+) regulatory T cells: their knowns and unknowns. Immunol Cell Biol. 2011;89(3):346–51.
Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity. 2009;30(6):899–911.
Nakayamada S, Takahashi H, Kanno Y, and O'Shea JJ. Helper T cell diversity and plasticity. Curr Opin Immunol. 2012.
Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155(3):1151–64.
Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;133(5):775–87.
Shevach EM. Suppressor T, cells: rebirth, function and homeostasis. Curr Biol. 2000;10(15):R572–5.
Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity. 2006;25(2):195–201.
Siddiqui KR, Powrie F. CD103+ GALT DCs promote Foxp3+ regulatory T cells. Mucosal Immunol. 2008;1(Suppl 1):S34–8.
Sojka DK, Huang YH, Fowell DJ. Mechanisms of regulatory T-cell suppression – a diverse arsenal for a moving target. Immunology. 2008;124(1):13–22.
Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. Nat Immunol. 2008;9(3):239–44.
Zhou X, Bailey-Bucktrout SL, Jeker LT, Penaranda C, Martinez-Llordella M, Ashby M, Nakayama M, Rosenthal W, Bluestone JA. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. Nat Immunol. 2009;10(9):1000–7.

Repertoire Selection

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Synonyms

Immunoglobulin repertoire

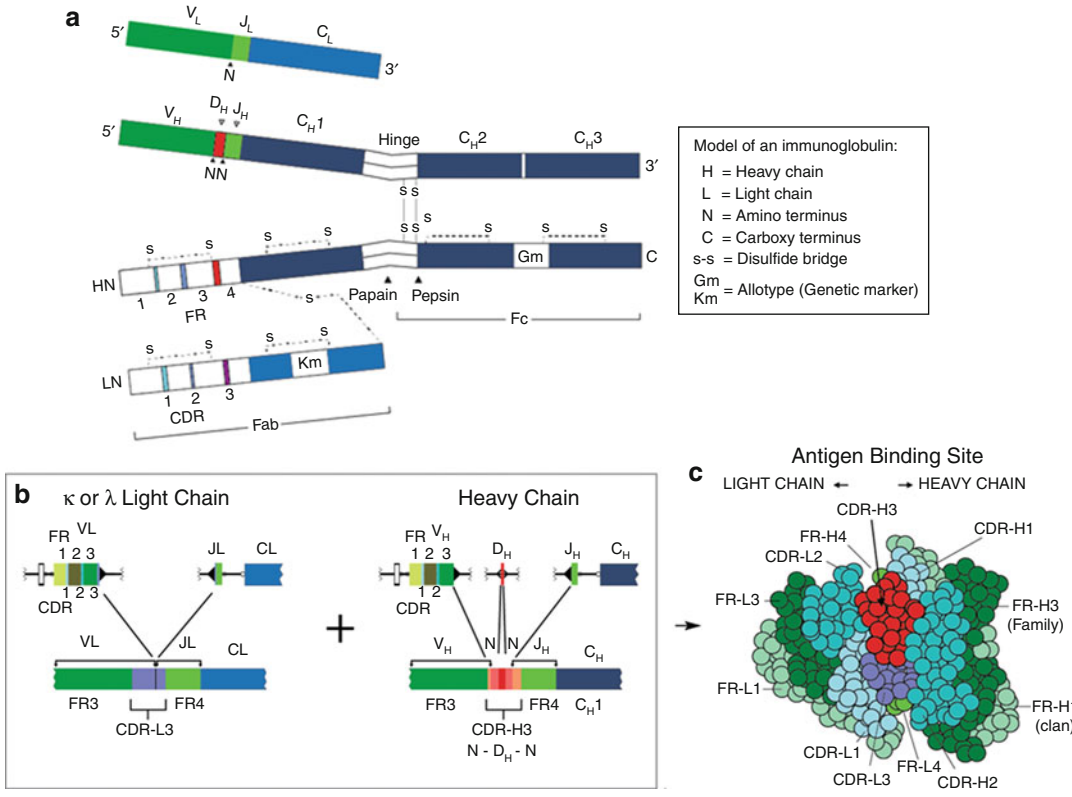
Definition

Repertoire selection is the process by which the antigen binding characteristics of the B cell receptor are used to maintain, activate, or expand B cell clones that make beneficial antibodies; and to suppress, sequester, or eliminate B cell clones that make ineffective or potentially pathogenic antibodies.

Introduction

Immunoglobulin (Ig), which in its membrane form functions as the B cell receptor (BCR) for antigen, is created by a complex process of clonal V(D)J rearrangement and N addition in developing B lineage cells. This process occurs in the bone marrow and fetal liver, both central lymphoid organs (Tonegawa 1983). Immunoglobulin belongs to the eponymous immunoglobulin superfamily (IgSF). They consist of two heavy (H) and two light (L) chains (Fig. 1), where the L chain can consist of either a κ or a λ chain (Schroeder and Cavacini 2010). Each component chain contains one NH_2-terminal "variable (V) IgSF domain and one or more COOH-terminal "constant" (C) IgSF domains, each of which consists of two sandwiched β pleated sheets "pinned" together by a disulfide bridge between two conserved cysteine residues. Each V or C domain consists of approximately 110–130 amino acids, averaging 12–13 kDa. L chains contain only one C domain, whereas H chains contain three or four. H chains with three C domains tend to include a spacer *hinge* region between the first (C_H1) and second (C_H2) domains. Considerable variability is allowed to the amino acids that populate the external surface of the IgSF domain and to the loops that link the β strands. These solvent-exposed surfaces offer multiple targets for docking with other molecules.

Following exposure to antigen in the periphery, B cells can induce further genetic modifications of their immunoglobulin through the process of somatic hypermutation (Kato et al. 2012). The collection of individual immunoglobulins is termed the antibody repertoire. As a result



Repertoire Selection, Fig. 1 *Immunoglobulin structure and derivation*. (**a**) Two-dimensional model of an IgG molecule. The H and L chains at the top deconstruct the antibody at a nucleotide level. The chains at the bottom deconstruct the protein sequence. (**b**) V(D)J rearrangement and N nucleotide addition create an extremely diverse Ig repertoire. (**c**) The antigen binding site is the product of a nested gradient of diversity. The view is looking into the binding site as an antigen would see the antigen binding site. This site is created by the juxtaposition of the three CDRs of the H chain and the three CDRs of the light chain. The VH domain is on the right side. The central location of CDR-H3, which due to N addition is the focus for repertoire diversity, is readily apparent

of these mechanisms of diversification, the antibody repertoire becomes large in size and extraordinarily diverse in content. The size and diversity of the repertoire creates immunoglobulins that have the potential to neutralize or protect against ancient or novel hazardous agents coming from inside or outside the body. Unfortunately, it also creates a repertoire with the potential to generate autoimmune disease (Durandy et al. 2013). This can occur when B cells produce Ig that binds inappropriately to self-antigens. Repertoire selection comprises a series of processes used by the immune system to control the composition of immunoglobulin in order to enhance protection and minimize the risk of pathogenic autoimmunity.

Darwinian Natural Selection of the Repertoire

The binding of a cell surface receptor to its ligand typically transduces a signal that affects the function or fate of the cell, including activation, mitosis, differentiation, or death. To maintain the specificity of these downstream effects, each receptor typically coevolves with its ligand. The B cell receptor would seem to be an exception because at first glance, its ligand (antigen) binding site appears to vary randomly in sequence and structure on a large scale (Tonegawa 1983). However, intra- and interspecies comparisons reveal evidence of conservation, or stereotypy, of germline VDJ

gene sequences and structures that may have a deterministic effect.

The term *natural selection* was used by Darwin to describe nature's analogy to *artificial selection*, a process by which animals and plants with traits considered desirable by human breeders are systematically favored for reproduction. Natural selection is considered to be the primary explanation for adaptive evolution. The structures encoded by the framework 1 (FR1) and framework 3 (FR3) of the V domain are more highly conserved within mammals than the constant domains, with certain FR3 structures conserved from shark to human (Kirkham and Schroeder 1994). Thus, the structures of these scaffolds for the antigen binding site appear to be preserved by natural selection.

Individual V gene sequences can be grouped into families sharing FR3's of similar sequence and structure. These stereotyped FR3's help shape and frame the antigen binding site (Kirkham and Schroeder 1994). V families can be grouped into clans based on the sequence and structure of FR1, which influences the nature of the elbow joint interaction between the V domain and the CH1 domain (Lesk and Chothia 1988). The antigen binding site is created by the juxtaposition of three hypervariable loops from the H chain, termed complementarity determining regions (CDRs), with three CDRs from the L chain. CDR1 and CDR2, which are completely encoded by the V gene segment and are juxtaposed to FR3, demonstrate conserved canonical structures with a nonrandom association of CDR1 structures with CDR2 structures (Chothia et al. 1992), influencing the binding properties of the outer edge of the antigen binding site. Even CDR-H3, which is created de novo by VDJ recombination and random N nucleotide addition, demonstrates evidence of stereotypy based on specific physicochemical properties that include enrichment for neutral amino acids such as tyrosine and depletion of highly charged amino acids (Ivanov et al. 2002).

Stereotypy in CDR-H3 is partly due to natural selection of JH and DH gene segment sequence coupled with control of DH reading frame usage (Schroeder et al. 2010). Thus, although the antibody repertoire is not identical between different individuals of the same or different species, there are physicochemical biases of sequence, structure, and hydrophobicity that are emphasized, and others that appear to be repressed. The sequence of individual germline-encoded V gene segments can also play a critical role in immune responses. Violation of these constraints can yield increased susceptibility to infectious agents.

Clonal (Somatic) Central Selection of the Repertoire

Generation of an immunoglobulin molecule in the bone marrow and fetal liver is typically viewed as a stepwise process that involves passage through a series of checkpoints that are used to test the stability of the H and L chains, and then the initial binding characteristics of the IgM they produce (Vale and Schroeder 2010). Passage through these checkpoints includes testing for physicochemical properties of the antigen binding site including hydrophobicity and structure. The initial requirement for the survival of a developing B cell is that it creates an H chain protein that can create a pre-B cell receptor in association with surrogate light chain (SLC), which consists of two L chain homologues termed VpreB and λ5. Failure of the H chain to bind a SLC results in cell death. Cells that survive this process repeatedly divide and then initiate L chain rearrangement. Production of a functional L chain permits creation of a complete IgM molecule by the immature B cell. Failure of the L chain to bind effectively to the H chain leads to additional rounds of VJ L chain rearrangement, with λ rearrangement typically occurring after κ rearrangements have failed. Immature B cells, which express only IgM on their cell surface, are tested for binding to self-antigens present in the serum or in their surroundings. Cells that bind too vigorously to self-antigens can be driven to apoptosis, become anergic and die by neglect, or return to a state where they undergo receptor editing, i.e., further rounds of L chain VJ

rearrangement or, less commonly, H chain V→V replacement until they create a more acceptable IgM.

Clonal (Somatic) Peripheral Selection of the Repertoire

B cells that survive this process leave the bone marrow or fetal liver and progress through transitional stages that precede their development into mature B cells, which circulate throughout the body (Vale and Schroeder 2010). The fate of these mature B cells depends both on antigen-by-antigen discrimination and on lineage. In the mouse, two lineages of B cells have been well-described. The B-1 lineage derives primarily from early fetal and neonatal progenitors and expresses a repertoire with fewer N nucleotides. Therefore, it is more highly dependent on germline sequence. B-1 cells pass through the spleen and most are then shunted into the peritoneal cavity where they will produce the majority of circulating IgM (Baumgarth 2011). This IgM is heavily enriched for natural autoantibodies that are polyreactive and bind with low affinity to many self-antigens, including altered self-antigens created by catabolism or environmental influences. These molecules aid in cellular homeostasis. Due to greater reliance on germline sequence, many of these B cells create antibodies with "public" antigen binding sites that can be found in many individuals of the same species.

Also present in the spleen are marginal zone (MZ) B cells which also tend to bind self-antigens with low affinity (Lopes-Carvalho et al. 2005). These cells are created by adult stem cells. Many of these cells produce immunoglobulins that bind common microbial constituents. They form an already active cohort of B cells that can respond to blood-borne microbial challenge very quickly. Among these MZ B cells, immunoglobulins bearing antigen binding sites with less common physicochemical properties, including a high frequency of hydrophobic or charged amino acids, are found more frequently. Both B-1 and MZ B cells tend primarily to produce IgM, and they typically do not require T cell help.

B cells in the follicles of the spleen, lymph nodes, and ectopic lymphoid aggregates associated with inflammatory responses typically require T cell help in order to develop into plasma cells or memory B cells (Shlomchik and Weisel 2012). High affinity for antigen or a high concentration of antigen permits follicular B cells to develop into short-lived plasma cells. A lower affinity or antigen concentration promotes creation and entry into a germinal center. In the germinal center, B cells undergo repeated cycles of antigen selection and somatic hypermutation, a process termed affinity maturation. Somatic hypermutation permits massive changes to the framework scaffolding that supports the antigen binding site as well as major changes to the structure and sequence of the antigen binding site itself.

Pathogenic autoreactive IgG antibodies are often highly mutated (Durandy et al. 2013). Engineering these molecules into a pre-mutated state often produces V domains that no longer bind the self-antigen, suggesting that somatic hypermutation has circumvented the mechanisms of somatic selection used to prevent initial production of autoreactive sequences. The mechanisms used to reduce the likelihood of the production of pathogenic autoantibodies by somatic hypermutation remain uncertain. It is possible that the less regulated structures created by ectopic lymphoid tissue that forms as a consequence of inflammation may be inherently less able to control repertoire diversification by hypermutation. These are areas of significant current scientific interest.

Biomarkers for the Evaluation of the Efficacy of Repertoire Selection

There are several means by which the efficacy of repertoire selection can be assessed. These range from gross measurements of antibody reactivity in the serum to single cell analyses.

Serologic analyses of autoantigen or antipathogen reactivities have a long history in clinical medicine. Examples of autoantigen reactivities include determination of dsDNA binding

titers or antinuclear antibody (ANA) titers in SLE, and of rheumatoid factor (RF) titers in rheumatoid arthritis (Riemekasten and Hahn 2005). Examples of anti-pathogen reactivities include anti-pneumococcal polysaccharide titers, anti-hemophilus influenza titers, and anti-influenza titers. However, these reactivities measure the final stage of antibody production and do not address particular stages where repertoire selection has acted.

Anti-idiotype or cross-reactive idiotype antibodies offer another means of testing where selection is occurring or has failed. For example, the V4-34 v_H gene segment contains a sequence in its frameworks and CDRs that facilitates binding to blood group antigens and other self-glycoproteins including CD45. An anti-idiotypic antibody, termed 9G4, exists that can stain cells using the V4-34 gene segment in its BCR. This antibody can be used in fluorescence-activated cell sorting studies to assess when use of this gene segment is suppressed. Some patients with autoimmune diseases fail to suppress use of this gene segment, which enables identification of the checkpoints at which tolerance has failed (Cappione et al. 2005). Labeled antigen can also be used to assess the frequency and subset of B cells with affinity for specific self- or auto-antigens.

High-throughput sequencing is a new tool that can be used to evaluate the actual composition of the repertoire in great detail (Weinstein et al. 2009). Sequencing studies, especially those focusing on sorted B cell subsets, have the potential to answer many questions about the precise details of repertoire selection. Coupling sequencing with single cell analyses makes it possible to directly link antigen specificity to molecular content (DeKosky et al. 2013). These types of studies will likely revolutionize our understanding of repertoire selection.

Cross-References

- ▶ Autoantibodies in Rheumatoid Arthritis
- ▶ Systemic Lupus Erythematosus, Autoantibodies

References

Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. Nat Rev Immunol. 2011;11:34–46.

Cappione 3rd A, Anolik JH, Pugh-Bernard A, Barnard J, Dutcher P, Silverman G, Sanz I. Germinal center exclusion of autoreactive B cells is defective in human systemic lupus erythematosus. J Clin Invest. 2005;115:3205–16.

Chothia C, Lesk AM, Gherardi E, Tomlinson IM, Walter G, Marks JD, Llewelyn MB, Winter G. Structural repertoire of the human VH segments. J Mol Biol. 1992;227:799–817.

Dekosky BJ, Ippolito GC, Deschner RP, Lavinder JJ, Wine Y, Rawlings BM, Varadarajan N, Giesecke C, Dorner T, Andrews SF, Wilson PC, Hunicke-Smith SP, Willson CG, Ellington AD, Georgiou G. High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire. Nat Biotechnol. 2013;31:166–9.

Durandy A, Cantaert T, Kracker S, Meffre E. Potential roles of activation-induced cytidine deaminase in promotion or prevention of autoimmunity in humans. Autoimmunity. 2013;46:148–56.

Ivanov II, Link JM, Ippolito GC, Schroeder Jr HW. Constraints on hydropathicity and sequence composition of HCDR3 are conserved across evolution. In: Capra JD, Zanetti M, editors. The antibodies. London: Taylor and Francis; 2002.

Kato L, Stanlie A, Begum NA, Kobayashi M, Aida M, Honjo T. An evolutionary view of the mechanism for immune and genome diversity. J Immunol. 2012;188:3559–66.

Kirkham PM, Schroeder Jr HW. Antibody structure and the evolution of immunoglobulin V gene segments. Semin Immunol. 1994;6:347–60.

Lesk AM, Chothia C. Elbow motion in immunoglobulins involves a molecular ball-and-socket joint. Nature. 1988;335:188–90.

Lopes-Carvalho T, Foote J, Kearney JF. Marginal zone B cells in lymphocyte activation and regulation. Curr Opin Immunol. 2005;17:244–50.

Riemekasten G, Hahn BH. Key autoantigens in SLE. Rheumatology (Oxford). 2005;44:975–82.

Schroeder Jr HW, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010;125:S41–52.

Schroeder Jr HW, Zemlin M, Khass M, Nguyen HH, Schelonka RL. Genetic control of DH reading frame and its effect on B-cell development and antigen-specifc antibody production. Crit Rev Immunol. 2010;30:327–44.

Shlomchik MJ, Weisel F. Germinal center selection and the development of memory B and plasma cells. Immunol Rev. 2012;247:52–63.

Tonegawa S. Somatic generation of antibody diversity. Nature. 1983;302:575–81.

Vale AM, Schroeder Jr HW. Clinical consequences of defects in B-cell development. J Allergy Clin Immunol. 2010;125:778–87.
Weinstein JA, Jiang N, White 3rd RA, Fisher DS, Quake SR. High-throughput sequencing of the zebrafish antibody repertoire. Science. 2009;324:807–10.

Resolution of Inflammation

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Synonyms

Catabasis; Lipid-mediator class switching

Definition

Resolution of inflammation is the termination of an inflammatory response.

Brief Overview

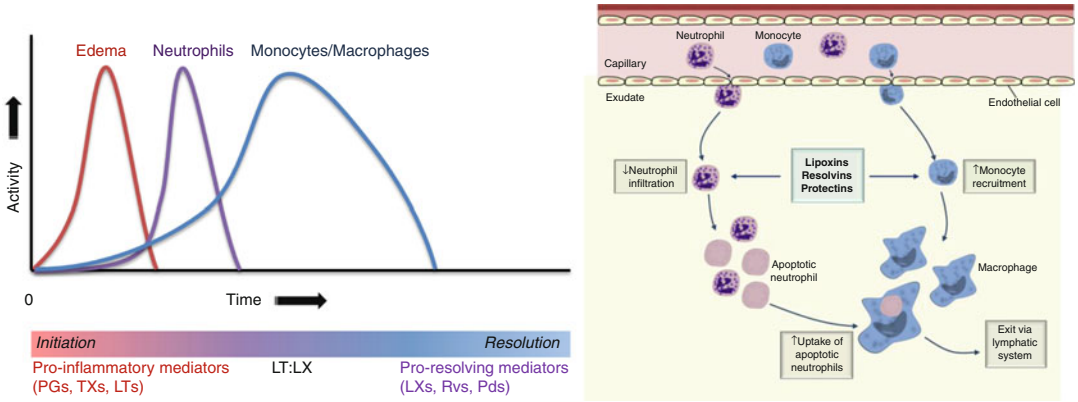
Acute inflammation is a protective response to invading microbes and tissue injury, and resolution is necessary for tissues to return to their normal state after the benefits of acute inflammation have been achieved. The resolution process is important for prevention of diseases characterized by chronic inflammation and tissue injury, such as atherosclerosis, cancer, inflammatory arthritis, and inflammatory bowel disease.

Resolution of inflammation is the coordinated, active termination of an inflammatory response. The term encompasses a switch in enzymatic activity and the biological actions of subsequently produced pro-resolving mediators, as well as the apoptosis and clearance of inflammatory cells (reviewed in Serhan 2007; Serhan et al. 2008). The process is typified by (1) the production of distinct lipid mediator classes: eicosanoids, resolvins, protectins, maresins, and anti-inflammatory cytokines; and (2) an active switch in cell types within the site of inflammation. Pro-resolution mediators signal termination of an inflammatory response by reducing ▶ neutrophil infiltration, activating ▶ neutrophil apoptosis, and recruitment of monocytes that mature into macrophages which clear apoptotic ▶ neutrophils and inflammatory debris. This process allows the tissue to return to homeostasis, a process also referred to as catabasis.

Temporal Actions and Lipid-Mediator Class Switching

Resolution of inflammation is characterized by an active switch in enzyme activity that produces the mediators predominating in inflammatory sites (Fig. 1). Initially, proinflammatory prostaglandins and leukotrienes activate and amplify inflammatory cascades. Next, prostaglandin (PG) E_2 and PGD_2, by inducing the production of key lipoxygenases, promote the synthesis of mediators that have both anti-inflammatory and pro-resolution activities. These include lipoxins, resolvins, protectins, and maresins (Fig. 1) (Serhan and Savill 2005). These families of specialized pro-resolution lipid mediators are not immunosuppressive, but instead function in the resolution of inflammation by activating specific mechanisms to promote homeostasis. Pro-resolution mediators stimulate and accelerate resolution by influencing the function of several cell types (Table 1). These lipid mediators provide potent signals that selectively stop ▶ neutrophil and eosinophil infiltration, stimulate non-phlogistic (noninflammatory) recruitment of monocytes, activate macrophage phagocytosis of microorganisms and apoptotic cells, and increase the exit of phagocytes from the inflamed site through the lymphatics.

Lipid-mediator class switching refers to the transition in enzyme activity from production of proinflammatory eicosanoids to pro-resolution lipid mediator classes (Fig. 1). During an inflammatory response, leukocytes rapidly biosynthesize PGs and leukotrienes via



Resolution of Inflammation, Fig. 1 Acute inflammation temporal actions. At the onset of inflammation, edema occurs by vascular leakage. Neutrophils appear first, followed by monocytes and macrophages. The switch in predominant cell types is driven by a shift in production of leukotrienes (*LT*) to the production of specialized pro-resolution lipid mediators including the arachidonic acid product, lipoxins (*LX*) (Adapted with permission, from Fredman and Serhan (2011) Biochem J. 437(2):185–97; and Serhan, Chiang, and Van Dyke (2008) Nat Rev Immunol. 8(5):349–61)

cyclooxygenase (COX) and 5-lipoxygenase (LOX) activity. As inflammation proceeds, the ▶ neutrophils stop producing chemoattractants (e.g., leukotriene B_4) and within hours begin to convert arachidonic acid into protective lipoxins (Fig. 1), thereby serving as agonists to actively terminate inflammation and promote resolution (Fredman and Serhan 2011). In contained exudates and inflamed tissues, both PGE_2 and PGD_2 have proinflammatory activities, but each can also promote a switch in the expression of biosynthetic enzymes produced by infiltrating ▶ neutrophils that changes their phenotype to a pro-resolution phenotype (Serhan and Savill 2005). The nonenzymatic degradation products of PGD_2 (i.e., 15-deoxy-delta-12,14-PGJ_2 and related cyclopentanones) can facilitate resolution by promoting leukocyte apoptosis and macrophage clearance from inflamed sites via inhibition of ▶ nuclear factor-κB (NFκB) activation.

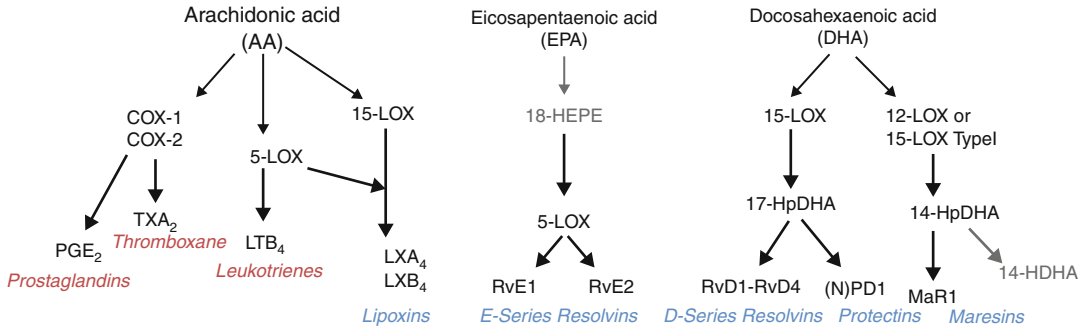
Lipoxins

Lipoxins, a large family of arachidonic acid metabolites, arise from the sequential action of 5-lipoxygenases and 15-lipoxygenases. Addition of 15-HPETE and 15-HETE to human leukocytes results in formation of a pair of oxygenated products containing a unique conjugated tetraene. One compound, lipoxin A4 (LXA4), was identified as 5,6,15L-trihydroxy-7,9,11,13 eicosatetraenoic acid, and the other proved to be its positional isomer (LXB4). Because both of these compounds can arise through an interaction between lipoxygenase pathways, the trivial name lipoxins (lipoxygenase interaction products) was introduced. Platelet 12-lipoxygenase can transform ▶ neutrophil leukotriene A4 (LTA4) to lipoxins. The complete stereochemistry and multiple routes of biosynthesis for the biologically active LXA4 and LXB4 – and their aspirin-triggered carbon 15-epimers (reviewed in (Serhan 2004)) – have been determined (Serhan et al. 2004). LXs generated by these cell–cell interactions stop ▶ neutrophil diapedesis and recruitment into the tissues and stimulate monocyte chemotaxis. LXs have additional effects on many cell types (Table 1).

In disease models, LXs and their aspirin-triggered epimers (stereoisomers that differ at only one site of chirality) have numerous effects (reviewed in Serhan et al. 2008). An abundance of literature documents the anti-inflammatory effects of LXs in rodent models of periodontitis, colitis, asthma, corneal disorders, glomerulonephritis, and pain.

Resolution of Inflammation, Table 1 Actions of specialized pro-resolution lipid mediators (Adapted with permission, from Serhan, Chiang, and Van Dyke (2008) Nat Rev Immunol 8(5):349–61)

Mediator	Target cell	Action(s)
Lipoxin A4 (*or aspirin-triggered lipoxin A4*)	Whole Blood leukocyte	Downregulates CD11b/CD18 expression
		Prevents shedding of L-selectin
		Reduces peroxynitrite generation on neutrophils, monocytes, and lymphocytes
	Neutrophil	Stops chemotaxis, adherence, and transmigration
		Stops neutrophil–epithelial cell and neutrophil–endothelial cell interactions
		Blocks superoxide anion generation
		Reduces CD11b/CD18 expression and $InsP_3$ formation
		Inhibits peroxynitrite generation
		Attenuates AP1, NF-κB accumulation, inhibits *IL8* gene expression
	Eosinophil	Stops migration and chemotaxis in vivo
		Inhibits generation of eotaxin and IL-5
	Monocyte	Stimulates chemotaxis and adhesion to laminin without increasing cytotoxicity
		Inhibits peroxynitrite generation
		Reduces IL-8 release by cells from individuals with asthma
	Macrophage	Stimulates non-phlogistic phagocytosis of apoptotic neutrophils
	T cell	Inhibits TNF secretion by blocking ERK activation
		Upregulates CCR5 expression
	Dendritic cell	Blocks IL-12 production
	Epithelial cell	Inhibits TNF-induced IL-8 expression and release in enterocytes
		Inhibits *Salmonella typhimurium*-induced IL-8 production by enterocytes
	Endothelial cell	Stimulates PKC-dependent prostacyclin formation
		Blocks the generation of reactive oxygen species
		Inhibits VEGF-induced endothelial cell migration
	Fibroblast	Inhibits IL-1β-induced IL-6, IL-8, and MMP3 production
		Inhibits CTGF-induced proliferation
Resolvin E1	Neutrophil	Stops transepithelial and transendothelial migration
	Macrophage	Stimulates non-phlogistic phagocytosis of apoptotic neutrophils
	Dendritic cell	Blocks IL-12 production
	T cell	Upregulates CCR5 expression
Resolvin D1	Microglia	Inhibits IL-1β expression
Aspirin-triggered resolvin D1	Neutrophil	Stops transmigration
Protectin D1	Neutrophil	Upregulates CCR5 expression
	Macrophage	Simulates non-phlogistic phagocytosis of apoptotic neutrophils
	T cell	Inhibits TNF and INFγ secretion and promotes apoptosis
		Upregulates CCR5 expression
	Microglia	Inhibits IL-1β expression
	Epithelial cell	Protects from oxidative stress-induced apoptosis in retinal pigment epithelial cells



Resolution of Inflammation, Fig. 2 Scheme of eicosanoid and specialized pro-resolution lipid mediators. *Red* indicates proinflammatory lipid mediators, and *blue* indicates pro-resolution lipid mediator classes (Reproduced with permission, from Fredman and Serhan (2011) Biochem J. 437(2):185–97)

Resolvins, Protectins, and Maresins

Resolvins, protectins, and maresins are synthesized from the marine-derived omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Fig. 2). These pro-resolution products help explain the mechanisms whereby omega-3 fatty acids – which predominate in oily fish – reduce the severity of inflammatory diseases.

Resolvins

Resolvins are derived from both EPA and DHA as the E-series and D-series, respectively. Like LXs, resolvins are formed by transcellular biosynthesis and include aspirin-triggered epimers that differ only in the stereochemistry at one carbon. EPA is converted to E-series resolvins (RvE1 and RvE2) via initial production of 18R-hydroeicosapentaenoic acid (18-HEPE) that is subsequently transformed by ► neutrophil 5-LOX. DHA is converted to D-series resolvins (RvD1 through RvD4) via the initial production of 17-hydroxyDHA (17-HDHA). Similar to the pro-resolution effects of LXA_4, resolvin E1 exhibits anti-inflammatory effects in rodent models of periodontitis, peritonitis, retinopathy, and colitis (Serhan et al. 2008). Measurable levels of RvE1 are produced in human plasma following acute intake of EPA with or without aspirin.

Protectins

The 17-hydroxyDHA derived from docosahexaenoic acid (DHA) can be converted into the 10,17-dihydroxy compounds known as protectins, which are characterized by conjugated triene double bonds. When produced in neural tissues, the prefix neuro- is added to denote the site of synthesis. Protectins exhibit pro-resolution effects in rodent models of peritonitis, asthma, ischemia–reperfusion injury, retinopathy, and ischemic stroke (Serhan et al. 2008). Reduced concentrations of DHA – and thus (neuro)protectin D1 – have been demonstrated in the brain tissues of patients with Alzheimer's disease.

Maresins

DHA is also converted by macrophage lipoxygenases to 14-hydroxyDHA (14-HDHA) and subsequently to dihydroxy products termed "macrophage mediators in resolving inflammation," or maresins (Serhan et al. 2009). Maresins have pro-resolving properties that are similar to resolvins and protectins.

Aspirin-Triggered Products

Aspirin acetylates COX-2 and causes it to act as a 15-LOX. The combined actions of 5- and 15-LOX result in aspirin-triggered epimers

(stereoisomers that differ only by chirality at one site) of lipoxins, resolvins, and protectins (Serhan et al. 2004; Spite and Serhan 2010; Oh et al. 2011). These compounds differ in their stereochemistry at one carbon: the aspirin-produced compounds have an "R" configuration in contrast to the "S" configuration of the non-aspirin-produced epimers. Production of aspirin-induced resolution products requires sufficient substrate availability (i.e., arachidonic acid, EPA, and DHA). Thus, intake of appropriate fatty acids is also necessary for the optimum benefits of low-dose aspirin used to prevent fatal cardiovascular events (Investigators 1999).

Implications for Disease and Therapeutics

Uncontrolled inflammation drives the pathogenesis of many diseases including arthritis, atherosclerosis, cancer, asthma, neurological disorders (e.g., Alzheimer's disease), periodontal disease, and inflammatory bowel disease. Lipoxins, resolvins, and protectins promote resolution in animal models of oral, lung, ocular, kidney, skin, and gastrointestinal inflammation, as well as in ischemia–reperfusion injury and angiogenesis (Serhan et al. 2008). For example, in a rabbit model of periodontitis, topical treatment of the gums with resolvin E1 prevents alveolar bone destruction and reduces ▶ neutrophil infiltration. Genetic experiments also demonstrate increased production of pro-resolution mediators in association with improved outcomes in several inflammatory conditions.

The multiple potent biological actions of specialized pro-resolution lipid mediators have implications for drug development because pharmacological therapies can interfere with or facilitate resolution. Glucocorticoids and aspirin may enhance resolution, whereas COX-2 inhibitors hinder it. In addition, direct administration of pro-resolution mediators or stable derivatives may promote resolution of inflammation in patients with conditions in which resolution is impaired (Filep et al. 1999). Adverse events due to pro-resolution therapy have not been observed in animal models, which is striking, given the problems associated with treatment with current anti-inflammatory agents.

References

Filep JG, Zouki C, et al. Anti-inflammatory actions of lipoxin A(4) stable analogs are demonstrable in human whole blood: modulation of leukocyte adhesion molecules and inhibition of neutrophil-endothelial interactions. Blood. 1999;94(12):4132–42.

Fredman G, Serhan CN. Specialized proresolving mediator targets for RvE1 and RvD1 in peripheral blood and mechanisms of resolution. Biochem J. 2011;437(2):185–97.

Investigators G-P. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet. 1999;354(9177):447–55.

Oh SF, Vickery TW, et al. Chiral lipidomics of E-series resolvins: aspirin and the biosynthesis of novel mediators. Biochim Biophys Acta. 2011; 1811(11):737–47.

Serhan CN. A search for endogenous mechanisms of anti-inflammation uncovers novel chemical mediators: missing links to resolution. Histochem Cell Biol. 2004;122(4):305–21.

Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol. 2007;25:101–37.

Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol. 2005;6(12):1191–7.

Serhan CN, Gotlinger K, et al. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. Prostaglandins Other Lipid Mediat. 2004;73(3–4): 155–72.

Serhan CN, Chiang N, et al. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008;8(5):349–61.

Serhan CN, Yang R, et al. Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. J Exp Med. 2009;206(1):15–23.

Spite M, Serhan CN. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. Circ Res. 2010;107(10):1170–84.

Rheumatoid Arthritis, Biologics in its Treatment

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Synonyms

Biologic disease-modifying antirheumatic drugs; Biological agents

Definition

Biological therapeutics described a family of protein-based therapeutic agents that are designed to specifically target cell membrane expressed, or soluble targets. They may comprise monoclonal antibodies (e.g., fully human, humanized, chimeric, murine), Fab fragments of monoclonal antibodies, or engineered soluble receptors usually with the addition of an Fc fragment. Further chemical modifications may facilitate altered half-life or tissue penetration/retention capabilities, e.g., pegylation.

Pathogenesis of RA: The Starting Point for Biologic Therapeutics

The advent of biologic therapeutic agents in RA has been defined by the remarkable progress in understanding of disease pathogenesis over the last 30 years. The synovial inflammation, bone erosion, and deformity seen clinically in RA represent the end-stage of an ill-defined interplay of the innate and adaptive arms of the immune response, together with complex interactions with stromal cells including fibroblasts, chondrocytes, osteoclasts, endothelial cells, and mesenchymal stem cells. Environment-gene interactions particularly smoking, together with epigenetic alterations and dysregulation of the gastrointestinal and oral microbiome, result in autoimmune responses against altered self-proteins arising from posttranslational modification of arginine to citrulline residues (McInnes and Schett 2011). This transitions to chronic inflammation, leading to articular damage and wider impact including increased vascular risk, depression, to disability, and to significant socioeconomic costs. Increasingly, the critical inflammatory pathways, and the cells that mediate checkpoints in this process, have been identified and in turn become the substrate as targets for a variety of biological therapies.

Background Therapeutic Approach to RA

While new biologic agents have mediated a step change in the treatment of RA, they should not obscure the key foundations of management. It is imperative that RA be treated by a rheumatologist and that decisions made are shared by the patient and aim for best care (Smolen et al. 2010).

In the 1980s, due in no small part to questionable benefits and toxic effects of the available drugs, the management of RA was based upon a concept of working slowly up a treatment pyramid until disease control was achieved. Non-pharmacological measures comprised the first step of management, accompanied by symptomatic relief through prescription of NSAIDs, followed eventually by "trial and error" based introduction of monotherapy, or combinations of titrating doses of an eclectic mix of immune modulatory medications – now referred to as conventional disease-modifying antirheumatic drugs (DMARD). These agents included methotrexate, sulphasalazine, hydroxychloroquine, gold, D-penicillamine and on occasion necessitated use of cytotoxic agents such as azathioprine or even cyclophophosphamide in severe and resistant disease. Outcomes were poor and medications associated with substantial toxicities and attendant morbidity.

The paradigm of management has completely shifted with the acknowledgement that active disease is a sign of ongoing bone erosion and that such bone damage is a significant prognostic factor for future disability. The new imperative is that remission, or at least low disease activity (however measured), is achieved as quickly as possible. Thus, the current concept is one of "inverting the pyramid" (van Vollenhoven 2009), i.e., that the most effective drugs are used quickly to try to establish early remission. Thus, treatment is now driven by early and aggressive intervention with frequent monitoring of therapy, encapsulated in the notion of "Treat to Target." The caveat to this approach is that not all patients with RA require the most potent agent available to induce remission, and a process in which every patient was immediately placed on biologics would potentially expose a significant group of patients to unnecessary toxic and expensive medications with no beneficence (Chatzidionysiou and van Vollenhoven 2011). Thus, new patients with active RA should be placed quickly on a DMARD and monitored frequently (every month if necessary) with escalation of treatment as required.

Methotrexate is the initial DMARD of choice and is considered the "anchor drug" of RA (Smolen et al. 2010). This is because no other DMARD has shown to be superior in effectiveness to it; its long-term and widespread use has resulted in a well-known and acceptable safety profile; and its use in combination with biologic agents is superior to the use of either alone. If there are contraindications/ intolerance of methotrexate, leflunomide, sulfasalazine, or injectable gold can be used as these agents are not known to be less effective than methotrexate. It should also be acknowledged that these three DMARDs can be used in conjunction with methotrexate to attempt to induce remission, but this attempt should not delay the introduction of biologic agents, and combination of DMARD treatments has not been shown to be any more effective than monotherapy while increasing the risk of adverse events. Antimalarial drugs have been shown to be less effective than the other DMARDs, but may be used in combination with other treatments or if the patient has very mild disease and contraindication to all other treatment options. Glucocorticoids have been shown to have an additive disease-modifying benefit and are usually used with DMARDs; however, the appropriate dosing is unknown and their long-term use may lead to significant side effects (Smolen et al. 2010).

It is recognized that RA probably encompasses several different diseases with similar clinical features but different prognoses and responses to treatment. Presence of autoantibodies, a high disease activity measured clinically or by CRP or ESR, and early appearance of erosions, all result in a worse prognosis (Emery et al. 2008). Patients in whom remission has not been achieved within 3–6 months and have these poor prognosis factors should be started upon a biologic agent in addition to methotrexate use, this being more effective than monotherapy with either drug. Without these prognostic factors, a second DMARD may be tried if methotrexate has not produced a response, with a biologic agent started if remission has not been achieved in a further 3–6 months. This approach, however, remains unsatisfactory as it potentially exposes those patients who do not achieve remission on DMARDs to up to 12 months of poorly controlled joint inflammation and damage, which results in an adverse prognosis (Chatzidionysiou and van Vollenhoven 2011). Thus, patients with very active disease, early structural damage, or unfavorable prognostic indicators may be considered for immediate first-line treatment with DMARDs and biologic agents (Emery et al. 2008); however, there is uncertainty whether this provides any more benefit than starting with monotherapy DMARD and switching after 3 months to combination therapy with a biologic agent. The early identification and decision over treatment of these patients is one of the current major rheumatology controversies and challenges.

TNF Inhibitors

The original and most frequent biologic agents licensed for use in RA are those that target TNF. TNF is one of the major drivers of synovial

inflammation: upregulating cytokine and chemokine expression, endothelial cell adhesion molecules, angiogenesis and pain, protecting synovial fibroblasts, and downregulating regulatory T cells (McInnes and Schett 2011). The five anti-TNF agents currently licensed are adalimumab, etanercept, infliximab, certolizumab, and golimumab. All Anti-TNF agents have been shown to be significantly superior clinically, functionally, and radiographically to placebo in patients who fail to respond to methotrexate. Importantly, combination of methotrexate and an anti-TNF agent is superior to either methotrexate or anti-TNF monotherapy. Anti-TNF monotherapy is clinically equivocal to methotrexate monotherapy, and although superior radiographically, still inferior to combination therapy.

The anti-TNF agents are now over a decade old and so have the best-defined safety profile; thus, they are usually the first biologic agent of choice. There have been few comparisons between biologic agents. One of the few trials comparing anti-TNF agents with other biologics, however, found that abatacept (see below) was at least as efficacious and offered a satisfactory safety profile. Due to lack of efficacy or intolerance, approximately one third of patients will discontinue their first biologic agent within a year. There is little evidence that increasing the dose of a TNF inhibitor leads to any significant clinical improvement, while it may increase the risk of adverse effects, the cost, and, in theory, can introduce delay until a patient achieves benefit from another agent. In contrast, there is evidence that switching between biologics of distinct mode of action is effective, and so this should be the next step in management once a patient fails their initial biologic agent. Some advocate that patients that fail a TNF inhibitor for an adverse event, or from gradual loss of effect (e.g., through anti-drug antibodies forming) may benefit from a trial of a second TNF targeting agent.

Other Available Biologic Agents

The focus on pathogenesis lead discovery has facilitated introduction of biologic agents that target other molecular pathways that are active in the RA lesion. Three other biologic agents are currently widely accepted, whereas a fourth, that targets IL-1 (anakinra), is now rarely used. Thus, tocilizumab, abatacept, and rituximab comprise the newest additions to the RA therapeutic armamentarium. All three agents have been shown to be significantly effective compared with placebo in patients who have not responded to anti-TNF therapy (van Vollenhoven 2009).

Tocilizumab is an antibody that binds the IL-6 receptor. IL-6 is the second major cytokine that has so far been successfully targeted in RA. It is responsible for activation of leucocytes and production of autoantibodies, while mediating systemic effects promoting the acute phase response, anemia, lipid metabolism dysregulation, and cognitive dysfunction (Chatzidionysiou and van Vollenhoven 2011). Tocilizumab mediates outcomes that are broadly similar to those achieved with TNF inhibitors in terms of magnitude and frequency of response. Some initial trials appear to indicate that it may induce a higher rate of remission compared with anti-TNF agents (Smolen et al. 2010); there is also now evidence that it may serve as well given as monotherapy as when administered with methotrexate.

Abatacept disrupts the interaction of the innate and adaptive immune systems as it contains a recombinant form of CTLA4 that prevents costimulation of the T cell during antigen presentation. T cells of type 17 and type 1 subset derivation are abundant in the synovial tissue of an inflamed joint, and T cells against citrullinated self-proteins have been described, suggesting that such pathways may be of pathogenetic importance (McInnes and Schett 2011). Mature T cells can exert cytotoxic effects, but more importantly, Th1 and Th17 cells further stimulate the innate immune response and host target tissues, e.g., fibroblasts, chondrocytes, and osteoclasts through release of cascades of pro-inflammatory cytokines. Abatacept appears to have a slower onset of action than anti-TNF agents, but seems to be clinically equivocal to the anti-TNF drugs in terms of magnitude and frequency of response.

Rituximab is an antibody against B cells, targeting CD20; administration in two pulses separated by 14 days results in effective B cell and plasmablast depletion. B cells are found in

T-cell-B-cell aggregates in the synovium, and their derivatives, plasma cells and plasmablasts, are found throughout the synovium and bone marrow (McInnes and Schett 2011). Although classically thought to be predominantly involved in inflammation through antibody formation, their more significant role in RA may be for autoantigen presentation and the production of inflammatory cytokines. Plasma cells are typically CD20 negative and as such, these cells are spared in rituximab treated patients. Rituximab appears to have similar clinical efficacy to the anti-TNF agents.

While anakinra, which binds the IL-1 receptor, was designed to be one of the biologic agents for treatment of RA, it has not shown a high level of clinical efficacy despite the prevalence of IL-1 in the inflammatory process. This is thought to probably be due to functional redundancy of IL-1 driven pathways in the autoimmune process. Anakinra is therefore not recommended as a major biologic option in RA.

Adverse Effects of Biologic Agents

The most common adverse effects of the biologic agents comprise injection site reactions or infusion reactions. More important from a clinical perspective, their effects on suppressing the immune system can result in an increased frequency of infections, particularly in the first 1–2 tears of receipt of therapy with a given agent. They are thus contraindicated in active infection, and there is relative contraindication for those with recurrent infections (Chatzidionysiou and van Vollenhoven 2011). In particular, the anti-TNF agents have been shown to reactivate TB (Gomez-Reino et al. 2003) and a range of other mycobacterial species with potentially lethal consequences – this has subsequently been demonstrated to be due to the dissolution of the granuloma that encases mycobacterial species within a granuloma in quiescent TB. Thus, it is vital that tuberculous prophylaxis be offered in patients with latent TB before any of the biologic agents are initiated. Rituximab, due to its effects on B cells, can lead to the reactivation of viral infections, including hepatitis.

Due to the suppression of the immune system, there is the theoretical risk that the biologic agents may increase the risk of malignancy by decreasing immunosurveillance and the destruction of neoplastic cells. Current malignancy remains an absolute contraindication, and prior malignancy is a relative contraindication – use of the biologics is sometimes considered depending on the severity of arthritis (Chatzidionysiou and van Vollenhoven 2011).

There is also the potential for the anti-TNF agents to trigger psoriasis, or more rarely SLE-like syndromes and very rarely, demyelinating disease. The anti-TNF agents and tocilizumab are thus contraindicated in patients with demyelinating disease, although rituximab and abatacept may be used. Rituximab has been shown to cause a small number of cases of progressive multifocal leukoencephalopathy. Due in part to IL-6's systemic effects, tocilizumab can cause several biochemical abnormalities, including neutropenia, thrombocytopenia, transaminitis, and hyperlipidemia, and has rarely been found to cause gastrointestinal perforations. The hyperlipidemia is an increase in total LDL and HDL cholesterol, and does not appear to change the atherogenic index, although its long-term implications are unknown (Chatzidionysiou and van Vollenhoven 2011).

There is little information surrounding the teratogenicity of biologic agents, so they are currently contraindicated in pregnancy and lactation. The biologic agents, except for abatcept, are also contraindicated in moderate-severe heart failure despite a paucity of good data (Chatzidionysiou and van Vollenhoven 2011).

When Biologic Agents Should Be Withdrawn?

Due to the adverse event potential of the biologic agents, their cost, and the principle that no patient should necessarily be reliant upon life-long medication, the ultimate aim of treatment should be drug withdrawal when safe and feasible. One of the major challenges in rheumatology today is to identify those patients in which this is a reasonable and safe approach.

Conclusion

Biologic agents have dramatically improved the treatment of RA over the last few years, but this should not obscure some of the other underlying principles of management nor the role that other medications have to play, especially in combination with biologic agents. It must also be acknowledged that although biologic agents have made the remission the target for many patients, the majority will not achieve this. With the introduction of new biologic agents, and more information about the use of current biologic agents, this target should be achievable for more patients. When a biologic agent should be started, and which patients should receive it earlier; which biologic agent should be started first, which should be switched to if this fails, which is most efficacious with the lowest adverse events, and does this change with different patients; and can a biologic agent be stopped, all these are just some of the challenges that the introduction of biologic agents has posed for the next few decades to come.

Cross-References

- ▶ Autoantibodies in Rheumatoid Arthritis
- ▶ Rheumatoid Arthritis, Clinical Features
- ▶ Rheumatoid Arthritis, Treatment

References

Chatzidionysiou K, van Vollenhoven RF. When to initiate and discontinue biologic treatments for rheumatoid arthritis? J Intern Med. 2011;269:614–25.

Emery P, McInnes IB, van Vollenhoven R, Kraan MC. Clinical identification and treatment of a rapidly progressing disease state in patients with rheumatoid arthritis. Rheumatology. 2008;47:392–8.

Gomez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: A multicenter active-surveillance report. Arthritis Rheum. 2003;48:2122–7.

McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365:2205–19.

Smolen JS, Landewe R, Breedveld FC, Dougados M, Emery P. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Ann Rheum Dis. 2010;69:964–75.

van Vollenhoven RF. Treatment of rheumatoid arthritis: state of the art 2009. Nat Rev Rheumatol. 2009;5:531–41.

Rheumatoid Arthritis, Clinical Features

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Synonyms

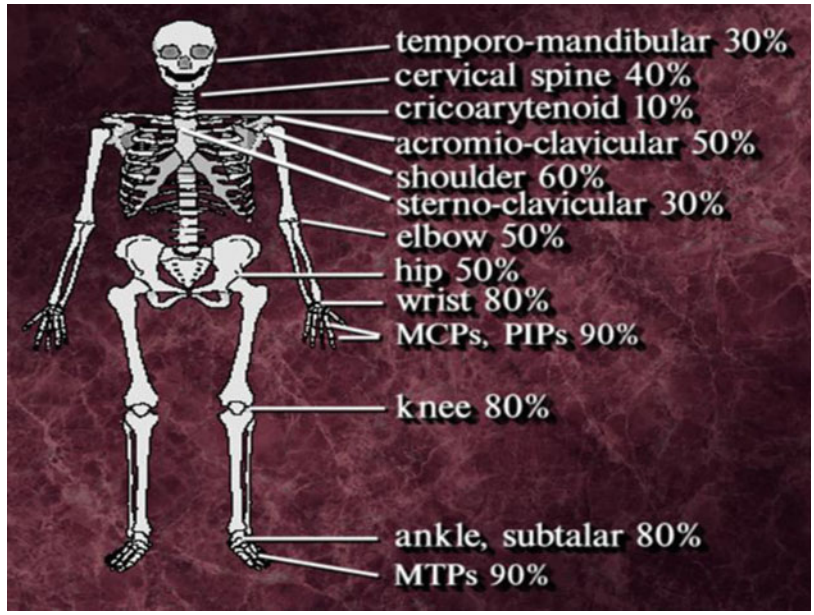
RA; Rheumatoid arthritis

Definition

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory disease of unknown etiology that affects the small and large joints in a symmetric fashion. Untreated, RA may lead to deformities and bony destruction of joints and the surrounding soft tissue structures in many patients. Subtypes of RA include patients who are seronegative for rheumatoid factor, lack joint erosions, and have a significantly better prognosis.

Introduction

RA is a systemic disease that predominately affects the joints but is a systemic illness leading to a number of extra-articular manifestations (see entry "▶ Rheumatoid Arthritis, Extra-articular Manifestations"). Patients with RA experience joint pain, swelling, stiffness, loss of mobility, decreased ability to perform activities of daily living (ADLs), as well as constitutional



Rheumatoid Arthritis, Clinical Features, Fig. 1 Joints distribution in RA. *MCP* metacarpophalangeal, *PIP* proximal phalangeal, *MTP* metatarsophalangeal

symptoms. This entry reviews the clinical features of this disease, particularly those affecting the joints and surrounding soft tissue structures.

Major Clinical Features

Constitutional Symptoms

Weakness, fatigue, weight loss, anorexia, and even low-grade fevers occur in patients with RA, particularly those with very active disease. These symptoms are nonspecific and can precede the onset of arthritis by months.

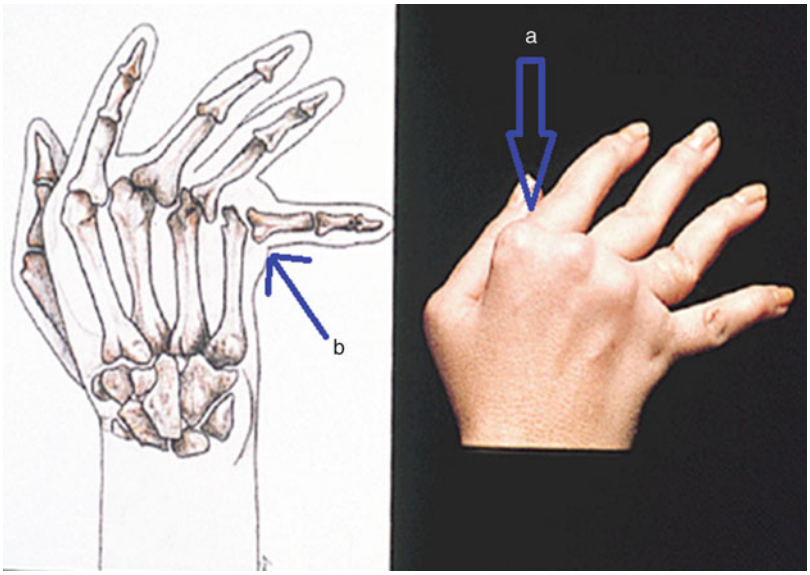
Stiffness

A hallmark feature of patients with active RA is generalized morning stiffness. Patients describe it as difficulty moving their joints after waking up, difficulty getting out of bed, and inability to start their morning activities. Morning stiffness in RA typically lasts for at least 1 hour when disease is active. This contrasts with the stiffness associated with osteoarthritis (OA), which is typically much more transient. Although it is rather subjective, the duration of morning stiffness may be useful in following RA disease activity (Lineker et al. 1999). Patients with RA also report "gelling" of their joints with inactivity, such as when sitting for an extended period of time. The key symptoms and physical signs based on the joints most commonly affected by RA will be described below.

Joint Manifestations

Rheumatoid arthritis typically affects peripheral diathrodial joints in a symmetric fashion. However, the symmetric joint involvement may be less evident early in the disease course.

RA may first manifest as a monoarthritis (involvement of one single joint), usually in a large joint. Monoarthritis tends to develop into polyarthritis within weeks or months, and ultimately RA affects small, medium, and large joints throughout the body (Fig. 1). RA causes pain, swelling, erythema (redness), and tenderness in the joints. The swelling occurs in the soft tissues around the joint (tenosynovitis) and/or in the joint space itself (synovium). Synovitis (inflammation of the normally thin lining of the joint) causes a "sponginess" or "bogginess" felt by the examiner and usually leads to pain when the joint is squeezed or moved. The joints also may feel warm on manual palpation. RA joint inflammation leads to a loss of mobility (range of motion) and a subsequent decline in ADLs and health-related quality of life. Although a trained



Rheumatoid Arthritis, Clinical Features, Fig. 2 Ligamentous laxity resulting in characteristic volar subluxation (*a*) and ulnar deviation (*b*) (© 2013 American College of Rheumatology. Used with permission)

examiner can usually identify joint swelling, tenderness, and painful range of motion, patients are often less accurate in differentiating joint swelling from tenderness, especially early in the disease course. This difficulty in differentiating pain alone from actual joint swelling (a hallmark of RA) often complicates or delays the diagnosis and assessment of disease activity. As RA progresses, particularly if inadequately treated, it may lead to fixed deformities in the joints.

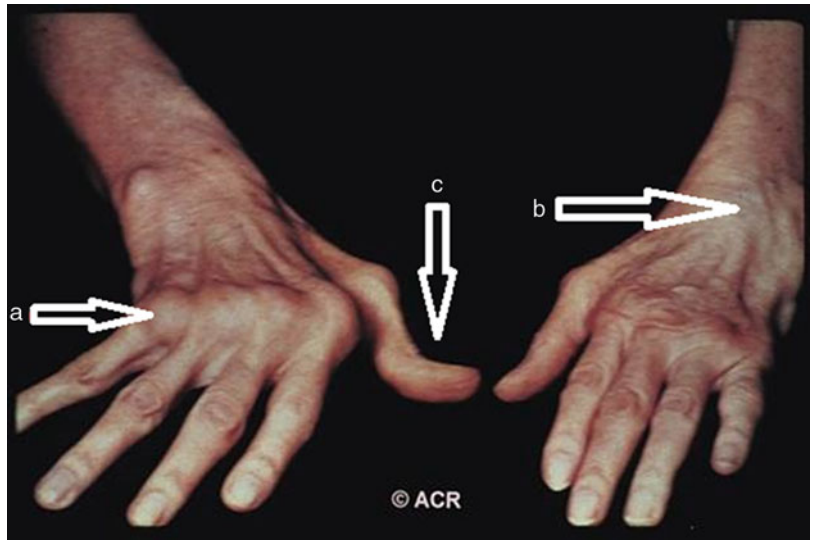
Hands and Wrist

The hands and wrists are affected in virtually all RA patients. The metacarpophalangeal (MCPs) and proximal interphalangeal (PIP) are involved in nearly all patients, whereas the distal interphalangeal (DIPs) joints are rarely involved. Swelling leads to diminished hand closure and grip strength coupled with pain and reduced ability to perform simple manual tasks such as eating, opening jars, and personal hygiene. Radial deviation at the wrist can occur with protracted inflammation. This is commonly associated with ulnar deviation of the digits (the metacarpal bones) (Fig. 2). This, along with palmar subluxation of the proximal phalanges, may lead to a "Z-deformity" of the thumb (Fig. 3). Other common deformities of the hands are volar (palmar) subluxation of the carpus bones on the wrist, bow string sign (prominence of the extensor tendons of the hands), swan-neck deformity of the digits (hyperextension of the proximal interphalangeal joints with compensatory flexion of the distal interphalangeal joints) (Fig. 4), boutonniere deformity (flexion contracture of the proximal interphalangeal joints and extension of the distal interphalangeal joints), and prominence of the ulnar styloid with hypermobility (piano key deformity).

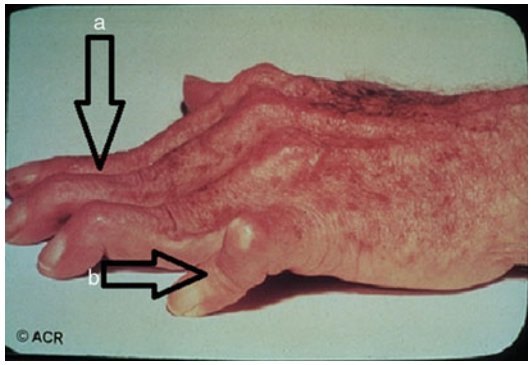
RA complications in the hands include (1) carpal tunnel syndrome (this results from synovitis in the wrist, causing the median nerve to be compressed and leading to symptoms of numbness and tingling in digits 1–3 (and the radial aspect of the 4th digit) and eventual weakness in the hand (affecting the thenar eminence and the thumb)) and (2) extensor tendon rupture (this occurs in patients with significant dorsal tenosynovitis). Radiocarpal subluxation and tendon rupture lead to inability to extend the fourth and fifth digits (Fig. 5) (Fleming et al. 1976).

Knees

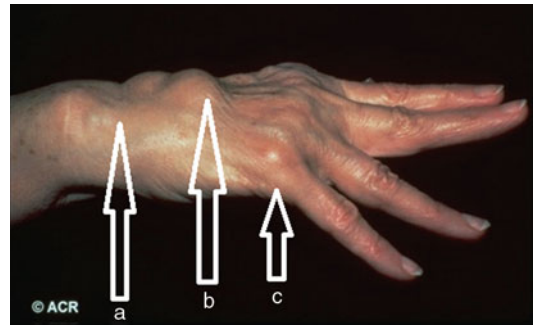
Inflammation of the knees causes swelling and pain. Knee symptoms are some of the most debilitating of RA due to their effects on standing and gait. Synovitis and a synovial joint effusion can usually be easily detected on physical



Rheumatoid Arthritis, Clinical Features, Fig. 3 Prominent ulnar deviation in the right hand, metacarpophalangeal (*MCPs*) (*a*), and proximal interphalangeal (*PIP*) swelling in both hands and synovitis of left wrist (*b*) and Z-deformity of the right thumb (*c*) (© 2013 American College of Rheumatology. Used with permission)



Rheumatoid Arthritis, Clinical Features, Fig. 4 Swan-neck deformity in digits 2, 3, and 4 (hyperextension of the proximal interphalangeal joints with compensatory flexion of the distal interphalangeal joints) (*a*) and boutonniere deformity in digit 5 (flexion contracture of the proximal interphalangeal joints and extension of the distal interphalangeal joints) (*b*) (© 2013 American College of Rheumatology. Used with permission)



Rheumatoid Arthritis, Clinical Features, Fig. 5 Radiocarpal volar subluxation (*a*) and dorsal tenosynovitis (*b*) leading to tendon rupture and an inability to extend the fourth and fifth digits (*c*) (© 2013 American College of Rheumatology. Used with permission)

examination and do not require advanced imaging in most cases. A fluid wave ("bulge sign") and a "ballotable" patella are key findings on the examination. A complication of a large or protracted knee effusion is a popliteal synovial cyst, also known as Baker's cyst. Baker's cysts manifest as swelling in the back of the knee and are not specific to RA. In RA, popliteal cysts occur secondary to knee joint inflammation and the popliteal space communicates with the articular space. Most popliteal cysts are small and asymptomatic, but they can lead to knee pain and stiffness and restricted mobility. If the cyst is big and compressing adjacent structures, it might resemble a deep venous thrombosis.

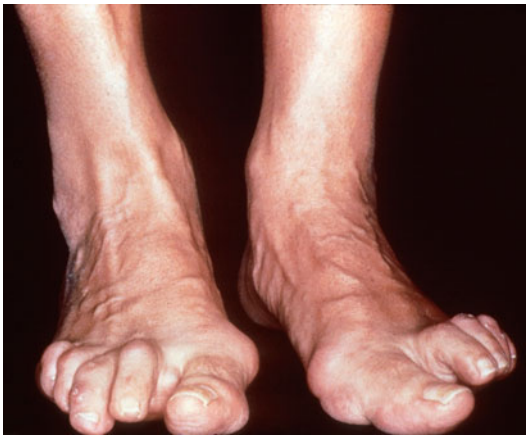
Feet and Ankles

Beyond the hands and knees, the small joints in the feet, particularly the metatarsophalangeal (MTP) joints, are affected in nearly all patients with RA (Fig. 1). This results in painful ambulation, and the sensation of "walking on marbles." MTP involvement can lead to "cock-up" deformities of the toes (Fig. 6). Other foot deformities include widening of the forefoot, hallux valgus,

lateral deviation, and dorsal subluxation at the toes (Fig. 7). The ankle and the talonavicular joints are also commonly affected in RA.

Hips

The hip joints are less commonly affected in RA than are the other large joints. When RA significantly affects the hip, it occurs later in the disease course. Earlier in RA, hip involvement is usually asymptomatic. An RA patient with significant hip involvement might experience groin pain, which further can be elicited on hip range of motion on physical examination (particularly a restriction in internal rotation). However, it is hard to detect synovial inflammation of the hip by physical examination alone.



Rheumatoid Arthritis, Clinical Features, Fig. 6 Cock-up toe deformities of the foot. (© 2013 American College of Rheumatology. Used with permission)

Shoulders

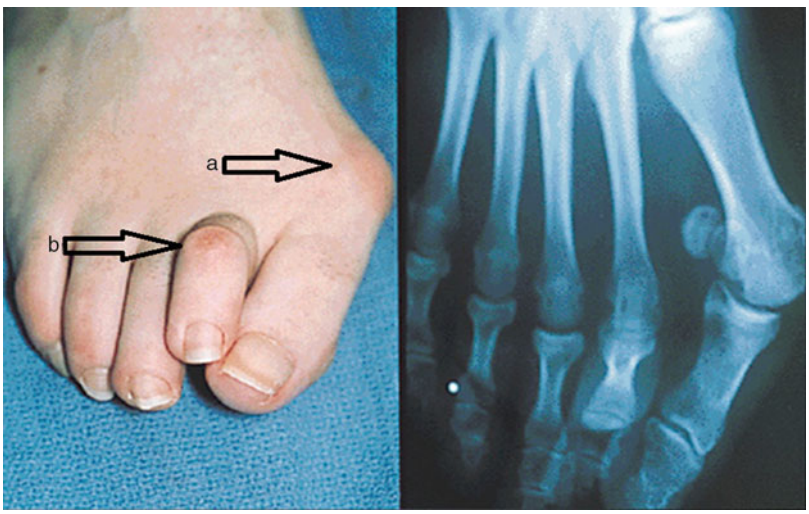
RA patients commonly experience pain and decreased range of motion in the shoulder region due to involvement of the glenohumeral joint. It is sometimes hard to appreciate a joint effusion on physical examination unless it is large, in which case a ballotable, anterior bulge may be appreciated.

Elbows

RA elbow involvement leads to pain, swelling, and loss of elbow flexion, extension, and pronation/supination. When the elbows are affected, it is usually appreciated on physical examination. Flexion deformities can develop later in the disease course and are manifest as inability to fully extend the elbows. Complications of RA elbow involvement include nerve entrapments around the elbow due to joint inflammation (leading to tingling, numbness, and weakness in the forearm and hand).

Cervical Spine

Axial skeletal involvement in RA is usually limited to the upper cervical spine. Typically, even moderate atlantoaxial RA is relatively asymptomatic and is seldom detected until neurologic



Rheumatoid Arthritis, Clinical Features, Fig. 7 Hallux valgus (first toe) (*a*) and hammer toe (second toe) (*b*). (© 2013 American College of Rheumatology. Used with permission)

symptoms develop (which may follow neck trauma). Symptoms of RA cervical involvement can include neck pain radiating to the occiput, sensory loss, and paresthesias (abnormal sensation such as tingling). Patients may describe a sensation of their head falling forward off their neck, if significant atlantoaxial laxity is present. RA neck disease is a potentially serious complication and it can lead to severe atlantoaxial subluxation (a misalignment of the first 2 cervical vertebrae). This complication can cause spinal cord compression (manifest as quadriparesis or paraparesis, sphincter dysfunction, sensory deficits, and/or transient ischemic attack due to compromise of the vertebral arteries) requiring immediate intervention (Neva et al. 2003).

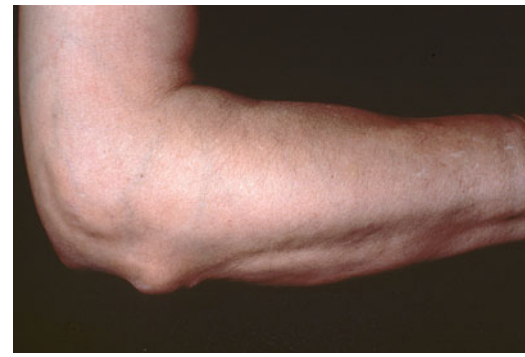
Extra-articular Manifestations

Some of the most common extra-articular manifestations include rheumatoid nodules (subcutaneous nodules that are common on pressure points) (Fig. 8), skin ulcers due to vasculitis (inflammation of the vessels), eye involvement (e.g., episcleritis or scleritis, both of which are considered medical urgencies), Sjogren's syndrome (manifest as dry eyes and dry mouth), and RA lung involvement (interstitial and pleural lung disease). The extra-articular manifestations are discussed in more detail elsewhere (Sayah and English 2005).

RA Comorbidities

There is an increased risk of serious comorbidities in patients with RA. The most common conditions leading to the comorbidity of RA are:

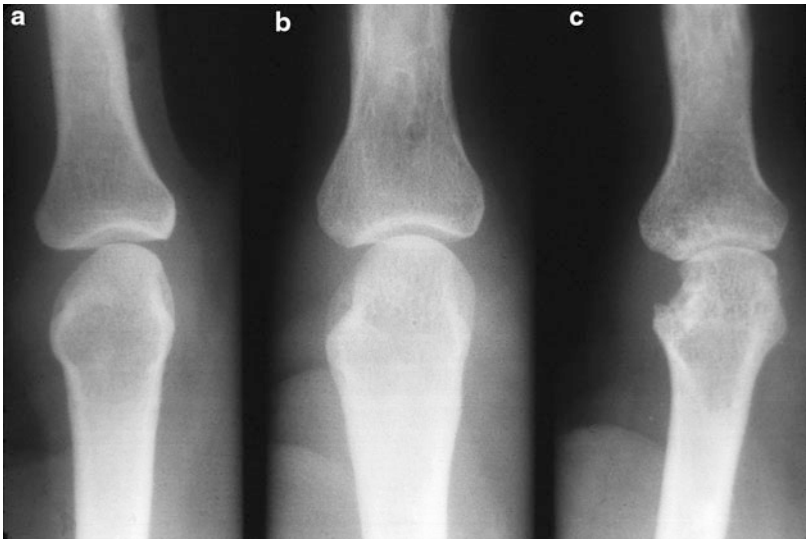
(a) Infection: there is an increased risk of infection in patients with RA mediated in part by use of immunosuppressive medication as well as from immunosuppression by the disease itself (Doran et al. 2002).
(b) Cardiovascular disease: there is an increased risk of cardiovascular disease in patients with RA, mostly related to RA disease activity and chronicity (Gabriel 2010). Use of glucocorticoids may also play a role.
(c) Renal disease: this is uncommon but includes secondary amyloidosis, membranous nephropathy, NSAIDs (nonsteroidal anti-inflammatory drugs)-induced nephrotoxicity, and focal mesangial proliferative glomerulonephritis (Helin et al. 1995).
(d) Lymphoproliferative disorders: there is an increased incidence of lymphoma and leukemia in patients with RA. The most common type of lymphoma is diffuse large B cell type (Baecklund et al. 2006).



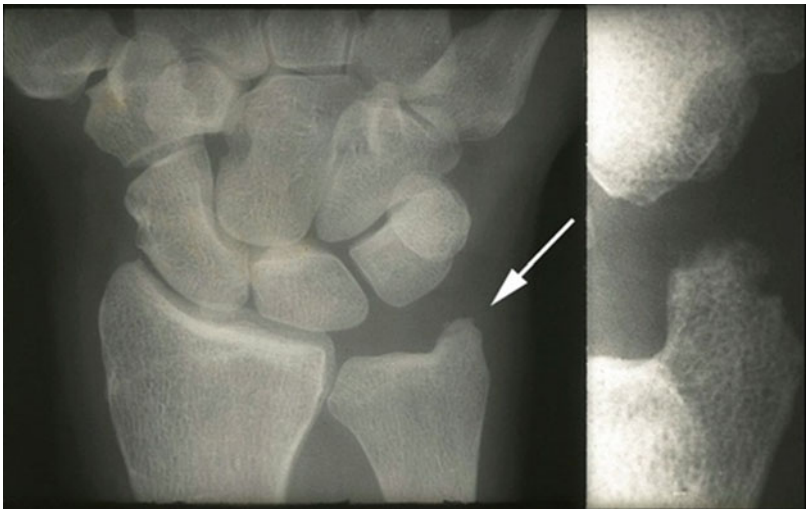
Rheumatoid Arthritis, Clinical Features, Fig. 8 Subcutaneous rheumatoid nodules at the elbow (© 2013 American College of Rheumatology. Used with permission)

Radiological Features

Early in the disease course, radiologic findings mostly reflect findings on physical examination: soft tissue swelling and joint effusions. With RA disease progression, radiologic findings include joint space narrowing due to loss of articular cartilage, which can be seen quite characteristically in the digits (Fig. 9), juxtaarticular osteopenia (loss of bone density around the joint), and bone erosions, which are usually marginal and can be seen in the digits (Fig. 9) and ulnar styloid (Fig. 10). Advanced RA disease in the hands is shown radiographically in Fig. 11. In the knees, cartilage loss affects both medial and



Rheumatoid Arthritis, Clinical Features, Fig. 9 (**a**) Soft tissue swelling. (**b**) Thinning of the radial side of the cortex with minimal joint space narrowing. (**c**) Marginal erosion appears on the radial aspect of the metacarpal head. There is loss of bone substance and joint space narrowing (© 2013 American College of Rheumatology. Used with permission)

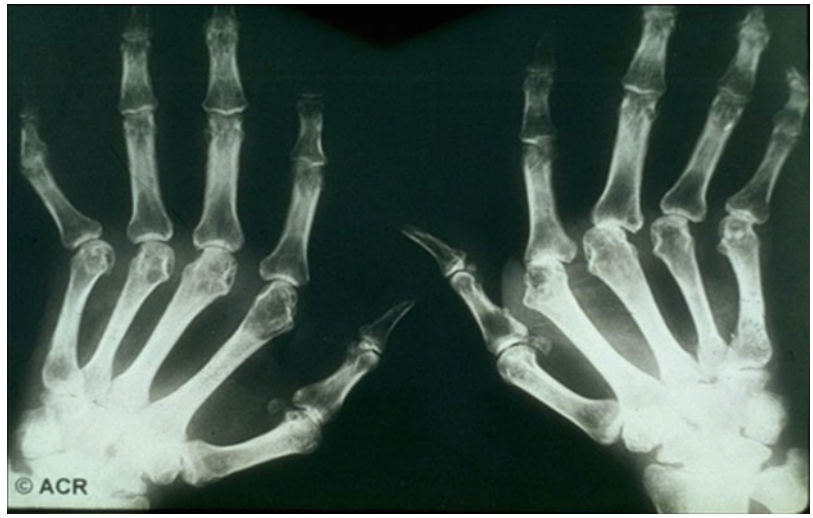


Rheumatoid Arthritis, Clinical Features, Fig. 10 Right-hand radiograph showing marginal erosions developing on the ulnar styloid (© 2013 American College of Rheumatology. Used with permission)

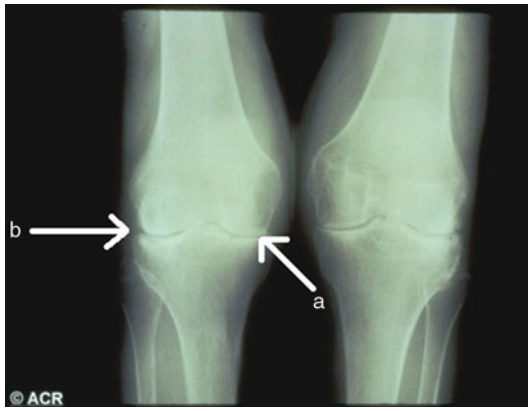
lateral compartments equally leading to joint space narrowing; this helps distinguish it from cartilage loss due to OA, which more commonly affects medial compartment first (Fig. 12). In the hip there is an axial or medial migration of the femoral head into the acetabulum. Atlantoaxial subluxation (described above) and cranial settling can be seen on a cervical spine radiograph (flexion/extension view), but cervical magnetic resonance imaging (MRI) or computerized tomography (CT) may be required to assess more carefully.

Classification Criteria

Because there is not one unique symptom, physical examination finding, or laboratory test that is the sine qua non of the RA diagnosis, classification criteria are useful for considering the key features of RA that constitute a case definition useful for clinical research studies. Although these criteria are largely for the purposes of clinical trials, they may also serve as a guide for clinical diagnosis. The American College of Rheumatology (ACR) and European League



Rheumatoid Arthritis, Clinical Features, Fig. 11 Radiograph of the hands showing advanced RA (© 2013 American College of Rheumatology. Used with permission)



Rheumatoid Arthritis, Clinical Features, Fig. 12 Knees radiograph showing medial (*a*) and lateral (*b*) compartment cartilage loss affecting both compartments of the knees equally, consistent with an inflammatory arthritis (© 2013 American College of Rheumatology. Used with permission)

Against Rheumatism (EULAR) developed revised criteria in 2010 for the classification of RA. In order to classify patients with RA, a patient should have at least one joint with synovitis (as long as there is no alternative explanation for the synovitis) and a total score of at least 6 out of 10 from four categories: joint number and distribution, serology, symptom duration, and acute phase reactants (Table 1) (Aletaha et al. 2010).

Differential Diagnosis

Many conditions should be considered in the differential diagnosis of RA. The most common is OA and factors that help differentiate RA from OA are listed in Table 2. Other conditions that may mimic RA and ways to distinguish them from RA (in parenthesis) include acute viral polyarthritis (most commonly due to rubella, parvovirus B19, HBV, and HCV and usually self-limiting), systemic lupus erythematosus (does not cause joints erosions and has more systemic extra-articular manifestations), inflammatory bowel disease-associated arthritis (patients have either Crohn's disease or ulcerative colitis and the arthritis typically affects the spine and sacroiliac joints, mostly large joints, and is often asymmetric), psoriatic arthritis (patients have psoriatic skin lesions, nail pitting, sausage digits, and sometimes spinal involvement), Lyme arthritis (a late manifestation of Lyme disease, usually presents as monoarthritis of the knee and typically does not cause joint destruction and there should be a history of tick exposure and an antecedent rash), gout and pseudogout (crystals are seen under polarized microscope in affected joints, serum urate is usually elevated, and polyarthritis is proceeded by a protracted period of episodic mono or oligoarthritis in gout), septic arthritis (usually monoarticular, especially

Rheumatoid Arthritis, Clinical Features, Table 1 The American College of Rheumatology (*ACR*) and European League Against Rheumatism (*EULAR*) 2010 RA classification criteria

	Points
Joint number and distribution	**(0–5)**
1 large joint	0
2–10 large joints	1
1–3 small joints (large joints not counted)	2
4–10 small joints (large joints not counted)	3
>10 joints (at least one small joint)	5
Serology	**(0–3)**
Negative RF and negative CCP	0
Low positive RF or low positive anti-CCP	2
High positive RF or high positive anti-CCP	3
Symptom duration	**(0–1)**
<6 weeks	0
≥6 weeks	1
Acute phase reactants	**(0–1)**
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1

Large joints: shoulders, elbows, hips, knees, and ankles; small joints: metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists; *RF* rheumatoid factor, *CCP* cyclic citrullinated peptide, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein

In order to classify patients with RA, they should have at least one joint with synovitis (as long as there is no alternative explanation for the synovitis) and a total score of at least 6 out of 10 points

involving the knee, with fevers, more significant constitutional symptoms, and commonly an infectious prodrome), and polymyalgia rheumatica (in older adults with acute and profound muscle pain in the shoulders and hip girdle) (Pease et al. 2009; Smith et al. 1987).

Assessment of Disease Activity

There are a variety of well-tested measures that are used in research and, increasingly, in clinical practice to quantitate the extent of disease activity and its response to treatment. Patients should be evaluated with the same disease activity measure at each visit. Some of these measures include (1) Disease Activity Score (DAS) 28 (this measure calculates the number of swollen and tender joints out of total 28 joints and uses either C-reactive protein or erythrocyte sedimentation rate in the calculation) (Table 3 shows DAS28 scores and corresponding disease activity), (2) Simplified Disease Activity Index (SDAI) (uses the C-reactive protein in the calculation), and (3) Clinical Disease Activity Index (CDAI) (a more simplified SDAI version and does not require an acute phase reactant in the calculation). The goal for each RA patient should be low disease activity or remission. In ideal

Rheumatoid Arthritis, Clinical Features, Table 2 Differentiating rheumatoid arthritis (*RA*) from osteoarthritis (*OA*)

	RA	OA
Age of onset	Starts in early adulthood; peak incidence within the fourth and fifth decades	Increases with age
Risk factors	HLA DR4, smoking	Trauma, sports injury, orthopedic procedures (post -arthroscopy), age
Early symptoms	Morning stiffness	Pain increases with use and as day progresses
Joints involved	MCPs, PIPs, wrists; not usually DIPs	DIPs, PIPs, base of thumb (CMCs), knees, hips, L-spine, C-spine
Physical exam findings	Soft tissue swelling, warmth, synovitis, tenderness	Osteophytes, bony enlargement, often nontender
Radiographic findings	Periarticular osteopenia, erosions	Osteophytes, subchondral sclerosis, asymmetrical joint space narrowing
Laboratory findings	Increase in ESR and CRP, rheumatoid factor (RF), white blood cell count, and anti-CCP antibodies; anemia	Normal results

HLA human leukocyte antigen, *MCP* metacarpophalangeal, *PIP* proximal interphalangeal, *DIP* distal interphalangeal, *CMC* carpal metacarpal, *ESR* erythrocyte sedimentation rate, *CCP* cyclic citrullinated peptide

Rheumatoid Arthritis, Clinical Features, Table 3 Disease Activity Score (*DAS*) 28 score and corresponding disease activity

	DAS28
High disease activity	>5.1
Moderate disease activity	3.2–5.1
Low disease activity	2.6–3.2
Remission	<2.6

The equation includes swollen joint count (0–28), tender joint count (0–28), erythrocyte sedimentation rate (*ESR*) or C-reactive protein (*CRP*), and visual analogue scale (*VAS*) for general health assessment (0–100 mm)
Joints involved in the equation are shoulders, elbows, wrists, metacarpophalangeal, proximal interphalangeal, and knees

circumstances, RA remission should be the target of therapy, but in others, low disease activity may be an acceptable target. A joint ACR/EULAR task force defined remission as a tender joint count, swollen joint count, C-reactive protein (mg/dl) level, and patient global assessment of $\leq$1 each or a simplified Disease Activity Score of $\leq$3.3 (Felson et al. 2011).

Prognosis

The course of RA is highly variable and difficult to predict. Historically, there has been an increase in mortality rate and a reduced median life expectancy of patients with RA if the disease is untreated, estimated at between 8 and 10 years of reduced life expectancy (Erhardt et al. 1989). RA itself accounts for 30 % of this increased mortality, while the remainder is due to other factors, particularly infection and gastrointestinal bleeding. If the disease is untreated, joint damage caused by RA can lead to functional disability and, in many cases, the inability to perform ADLs or work tasks.

The presence of one or more of the following implies a poor prognosis: (1) the presence of >20 inflamed joints, (2) radiographic evidence of bone erosions, (3) rheumatoid nodules, (4) high titers of serum rheumatoid factor or anti-CCP, and (5) older age (Saag et al. 2008). Additional factors that also have been linked to worse outcomes defined by early disability and morbidity include (1) female sex, (2) presence of the shared epitope (HLA DR-B1), (3) poor results on the Health Assessment Questionnaire (HAQ), and (4) cigarette smoking. A plethora of effective treatment options (see entry "▶ Rheumatoid Arthritis, Treatment") has significantly modified the natural history of RA leading to improved short- and longer-term outcomes.

References

Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham 3rd CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010; 62(9):2569–81.

Baecklund E, Iliadou A, Askling J, Ekbom A, Backlin C, Granath F, et al. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. Arthritis Rheum. 2006; 54(3):692–701.

Doran MF, Crowson CS, Pond GR, O'Fallon WM, Gabriel SE. Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. Arthritis Rheum. 2002; 46(9):2287–93.

Erhardt CC, Mumford PA, Venables PJ, Maini RN. Factors predicting a poor life prognosis in rheumatoid arthritis: an eight year prospective study. Ann Rheum Dis. 1989;48(1):7–13.

Felson DT, Smolen JS, Wells G, Zhang B, van Tuyl LH, Funovits J, et al. American College of Rheumatology/European League Against Rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. Arthritis Rheum. 2011;63(3):573–86.

Fleming A, Crown JM, Corbett M. Early rheumatoid disease. I. Onset. Ann Rheum Dis. 1976;35(4):357–60.

Gabriel SE. Heart disease and rheumatoid arthritis: understanding the risks. Ann Rheum Dis. 2010;69 Suppl 1: i61.

Helin HJ, Korpela MM, Mustonen JT, Pasternack AI. Renal biopsy findings and clinicopathologic correlations in rheumatoid arthritis. Arthritis Rheum. 1995;38(2):242–7.

Lineker S, Badley E, Charles C, Hart L, Streiner D. Defining morning stiffness in rheumatoid arthritis. J Rheumatol. 1999;26(5):1052–7.

Neva MH, Isomaki P, Hannonen P, Kauppi M, Krishnan E, Sokka T. Early and extensive erosiveness in peripheral joints predicts atlantoaxial subluxations in patients with rheumatoid arthritis. Arthritis Rheum. 2003;48(7):1808–13.

Pease CT, Haugeberg G, Montague B, Hensor EM, Bhakta BB, Thomson W, et al. Polymyalgia rheumatica can be distinguished from late onset rheumatoid arthritis at

baseline: results of a 5-yr prospective study. Rheumatology (Oxford). 2009;48(2):123–7.
Saag KG, Teng GG, Patkar NM, Anuntiyo J, Finney C, Curtis JR, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. Arthritis Rheum. 2008; 59(6):762–84.
Sayah A, English 3rd JC. Rheumatoid arthritis: a review of the cutaneous manifestations. J Am Acad Dermatol. 2005;53(2):191–209; quiz 10–2.
Smith CA, Woolf AD, Lenci M. Parvoviruses: infections and arthropathies. Rheum Dis Clin North Am. 1987;13(2):249–63.

Rheumatoid Arthritis, Extra-articular Manifestations

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Synonyms

RA complications; RA extra-articular features; RA extra-articular signs; Extra-articular involvement of RA; Nonarticular manifestations of RA, Systemic manifestations of RA

Definition

The term "extra-articular manifestations of rheumatoid arthritis" refers to involvement with disease of tissues and organs outside the joints. It is believed that the same or similar cytokine pathway that drives synovitis may be the culprit for the involvement of extra-articular structures. Extra-articular involvement usually occurs in active, severe, and long-standing articular involvement.

Introduction

Rheumatoid arthritis (RA) is an inflammatory disease manifesting primarily with joint inflammation which if not treated early and aggressively leads to articular destruction and decreased functionality.

Less commonly RA is complicated by involvement of organ systems other than the skeletal. Most organ systems may be affected to the degree that RA could be viewed not as a "purely" joint disease but as a multisystem entity. Some of these extra-articular manifestations have a significant impact on overall prognosis, are associated with more active articular disease, and correlate with worse morbidity and higher mortality.

Traditional reference textbooks would provide an extensive list of extra-articular RA manifestations spanning from most common to the rare ones. In this entry, an exhaustive description of each individual extra-articular manifestation reported in the literature will not be attempted. Instead, the most frequently encountered and the ones associated with grave prognosis and worse overall outcomes will be described. While overall the occurrence of extra-articular manifestations has not declined in the recent years (Turesson et al. 2004), there is evidence that more aggressive therapy of rheumatoid arthritis may have contributed to a lower incidence of the most serious ones (Bartels et al. 2010).

Rheumatoid Nodules

Rheumatoid nodules are the most common cutaneous manifestation in patients with rheumatoid arthritis (Turesson and Jacobsson 2004). They manifest as subcutaneous firm growths on the extensor surface of extremities or joints (olecranon, extensor surface of forearm, achille tendon) and at pressure points (sacrum or occiput). Contrary to tophaceous growths, they are attached to the underlying periosteum. Rheumatoid nodules are almost exclusively encountered in patients with seropositive disease and associate with active erosive disease, smoking, and a higher likelihood of development of other extra-articular manifestations (Nyhall-Wahlin et al. 2011). Occasionally, they can appear in internal organs such as the lungs but also in other tissues (heart, meninges, and larynx).

Pathologically, they represent granulomata with fibrinoid necrosis in the center – frequently with evidence of small vessel vasculitis – surrounded by palisading macrophages and lymphocytes (Palmer et al. 1987).

Treatment with methotrexate – and less frequently other DMARDs – can occasionally be associated with new nodules or an increase in number and/or size of nodules –a condition called methotrexate-induced accelerated nodulosis – but a causality link has not been established with certainty (Patatanian and Thompson 2002). It can occur even after response of polyarthritis to therapy and also in seronegative patients (Kerstens et al. 1992).

Hematologic Abnormalities

Anemia of chronic disease – also referred to as "anemia of (chronic) inflammation"– is frequently encountered in patients with active articular disease along with frequent reactive thrombocytosis. Both abnormalities can improve with treatment of joint disease with anti-inflammatory and disease-modifying anti-rheumatic drugs (DMARDs).

Leucocytosis may accompany steroid treatment or be indicative of underlying infection in this frequently immunocompromised population. Cytopenias may represent bone marrow suppression due to DMARD treatment.

Felty's syndrome consists of neutropenia usually with detectable splenomegaly and occasionally accompanied by lower extremity skin ulcerations or concurrent thrombocytopenia. It is only rarely encountered – less than 1% – and is usually seen in long-standing seropositive nodular erosive disease (Goldberg and Pinals 1980) and much less frequently at RA presentation or with inactive or "burned out" disease (Balint and Balint 2004). It is associated with a higher risk for the development of other extra-articular manifestations (Goldberg and Pinals1980). The major concern when Felty's syndrome occurs is infections due to neutropenia.

Multiple possible mechanisms may participate in the pathogenesis of neutropenia in Felty's syndrome ranging from impaired granulopoiesis (due to antibody-mediated inhibition of granulocyte colony–stimulating factor, a maturation arrest in the bone marrow) to increased destruction of neutrophils in the periphery (due to immune complex–mediated reduced survival of neutrophils, or destruction via anti-granulocyte antibodies and sequestration in the spleen) (Rosenstein and Kramer 1991). Bone marrow examination usually demonstrates normal myeloid cellularity with maturation arrest in most of the cases (Rosenstein and Kramer 1991). HLA DR4 antigen is present in higher frequency in patients with Felty's syndrome than it is for the general RA population (Dinant et al. 1980).

Treatment for Felty's syndrome consists of treatment with DMARDs in the same manner active joint disease would be treated, which in most of the cases is simultaneously present with Felty's. Methotrexate is the most frequently used agent while other agents have been used such as leflunomide, sulfasalazine, plaquenil, or gold (Rashba et al. 1996). Steroids have and can be used but the concern of increasing the risk of an infection or further immunosuppressing already infected patients may complicate the therapeutic approach. In cases of significantly low neutrophils and fear of or recurrent infections, granulocyte colony–stimulating factor (G-CSF) can be attempted (Krishnaswamy et al. 1996) although reports mention an increased risk for a flare in articular disease. Resistant cases can be treated with splenectomy. Newer biologic therapies have been tried in case reports and series but have not been proven to be consistently effective (Narvaez et al. 2012).

Large granular lymphocyte syndrome (LGL) is an entity with several similarities to Felty's syndrome– sometimes called pseudo-Felty's syndrome – but also with distinct differences. In LGL, an expansion of a clonal cytotoxic T-lymphocyte – or less commonly natural killer cells (Loughran et al. 1993) – expressing a distinct set of surface markers is present and is accompanied by neutropenia and splenomegaly. There is peripheral lymphocytosis in contrast to Felty's syndrome, and frequently, the total WBC count is normal. The bone marrow is infiltrated

with lymphocytes, and anemia and thrombocytopenia are common. LGL is not associated with erosive arthritis or other extra-articular manifestations, and it frequently presents concurrently with RA. Due to neutropenia, recurrent infections are common. Patients may die from lymphoproliferative disease/leukemia (Lamy et al. 2003). While splenectomy is a therapeutic modality for Felty's syndrome, it can exacerbate LGL. Treatment for LGL syndrome is otherwise similar to this for Felty's syndrome (Rosenstein and Kramer 1991).

Patients with RA are at increased risk for lymphoproliferative malignancies and in particular non-Hodgkin's lymphoma – most commonly large-cell B-cell lymphoma – compared to the general population (Wolfe et al. 2004). The question as to whether the disease itself or treatment with nonbiologic or biologic DMARDs is responsible for this increased risk is still being debated (Wolfe et al. 2004).

Eye Involvement

The most frequent manifestation of RA in the eye is xerophthalmia resulting in keratoconjunctivitis sicca. Patients complain of dry eyes, a sensation of foreign body in the eye, burning, and possibly photophobia. The severity of isolated dry eyes does not seem to correlate with RA disease activity. However, secondary Sjogren's syndrome (sicca accompanied by xerostomia) is most frequently encountered in patients with active uncontrolled RA (Fujita et al. 2005).

Diagnosis of sicca is assisted by slit lamp examination and a Schirmer's test. In cases where keratoconjunctivitis sicca is not associated with active RA, symptomatic treatment with artificial tears or oral cholinergic/muscarinic agents is beneficial. Topical cyclosporine is also used although it may take several weeks before the effect becomes apparent (Barber et al. 2005). In resistant cases, punctal occlusion is offered as a therapeutic option. In the cases of secondary Sjogren's associated with active RA, treatment directed to controlling synovial inflammation may offer control of xerophthalmia symptoms.

Episcleritis presents in less than 1 % of the RA patients and manifests with a red eye accompanied by a dull orbital ache but usually with preserved vision. In contrast with scleritis – described below – episcleritis is a benign and usually self-limited entity which may be treated with topical or oral anti-inflammatory agents or steroids.

Scleritis is seen less commonly but is a serious ocular manifestation associated with grave prognosis as it may threaten the vision. It is usually associated with uncontrolled articular inflammation and sometimes with the coexistence of other extra-articular manifestations including vasculitis (Watson and Hayreh 1976). It frequently presents bilaterally which is in contrast with scleritis in the general non-RA population (Fujita et al. 2005). Patients have severe pain, a red eye, and photophobia. If left untreated, scleritis may lead to severe damage of the sclera and scleromalacia. Treatment for scleritis consists of topical anti-inflammatory and steroid agents in combination with systemic steroid-sparing agents. For mild cases, oral NSAIDs can be used but frequently an increase in the dose of methotrexate or initiation of more potent agents such as cyclophosphamide with high dose steroids may be needed. Multiple other agents have been employed in the treatment of scleritis including the newer biologic agents such as TNF-α inhibitors (Theodossiadis et al. 2007).

Less frequent eye manifestations comprise a wide spectrum of diseases including uveitis, ulcerative keratitis, scleromalacia perforans (a condition of necrotizing scleritis without inflammation which leads to thinning of the sclera without symptoms or redness), and also complications relating to treatment with hydroxychloroquine which can cause both retinopathy and keratopathy or steroids which may increase the intraocular pressure leading to glaucoma or cataracts.

Vasculitis

Rheumatoid vasculitis (RV) is the most serious extra-articular manifestation of RA. In fact

a vasculitic process is assumed to possibly underlie several other extra-articular manifestations such as the rheumatoid nodules and scleritis/episcleritis.

While early autopsy investigations reported the prevalence of RV to more than 15% and the lifetime risk to 2% (Suzuki et al. 1994), more recent evidence shows a decline in incidence especially after the year 2000 (Bartels et al. 2009), suggesting an association with the availability of newer agents to treat RA and the overall trend to treat articular disease more aggressively.

RV is associated with RA of long duration, severe destructive disease, seropositivity for rheumatoid factor and/or anti-CCP, and also nodules, certain HLA alleles, and smoking (Voskuyl et al. 1996).

Small and medium vessels may be affected and biopsy shows leucocytoclasis or pauci-immune lesions. Essentially, every tissue may be affected, but most commonly the skin and peripheral nerves are targeted in RV. Mesenteric, cardiac, and renal involvement is rare. Patients may present with systemic symptoms of fatigue, fever, and weight loss, but concurrent active articular disease may confound the association of these symptoms with underlying or evolving vasculitis. Skin involvement may be in the form of palpable purpura or more commonly manifest as nail-fold infarcts and leg ulcerations. Occasionally digital ischemia leading to gangrene may be seen. A sensory or mixed sensory motor neuropathy indicates peripheral nerve involvement. Mononeuritis multiplex is a common presentation and patients may develop a foot or wrist drop in an acute fashion.

A solid diagnosis will require a skin or nerve biopsy to exclude atheromatous disease accounting for ischemia or extremity ulcerations, or other conditions such neutrophilic dermatoses that may also be encountered in patients with RA and account for ulcerations.

Treatment is usually tailored to the severity of RV. Traditionally nail-fold infarcts have been considered a benign manifestation which may respond to accelerated DMARD treatment for the underlying articular disease. Small skin ulcerations that do not expand may also be treated with low dose prednisone or DMARDs. However, digital ischemia and peripheral nerve involvement are considered more ominous manifestations that will require high dose steroids and usually cyclophosphamide. Newer biologic agents have also been reported to be beneficial in the treatment of rheumatoid vasculitis (Puechal et al. 2008).

Recently, an association of vasculitis with treatment with TNF-α inhibitors has been reported in case reports and case series. In most cases, vasculitis responded to discontinuation of the TNF-α inhibitor therapy and in some cases recurred upon reinstitution of treatment (Ramos-Casals et al. 2007; Guignard et al. 2707). In most of the reported cases, only the skin was involved. However, other reports show that TNF-α inhibitors have been used successfully to treat RV (Puechal et al. 2008). Whether there is indeed an increased risk for development of vasculitis after treatment with TNF-α inhibitors and the role of these agents in the treatment of RV is an area of uncertainty.

Cachexia: Body Composition Changes

RA is associated with altered anthropometric characteristics. A low BMI – in the underweight range – is common in patients with RA, with some studies reporting a prevalence of more than 10% (Munro and Capell 1997). A lower BMI has been associated with radiologic progression and higher levels of disease activity (Westhoff et al. 2007). This association of low BMI with adverse outcomes in RA has in fact been reported to be stronger than that of the shared epitope with the same outcomes (Kaufmann et al. 2003). A lower BMI has also been associated with higher risk for disability, all cause mortality and CV associated mortality (Summers et al. 2008).

Rheumatoid cachexia is a state of loss of lean body mass and is reported to occur in up to 50% of patients (Summers et al. 2008). Cachectic obesity is a condition – also commonly encountered in RA – in which loss of lean body mass is

accompanied by gain in adipose tissue, thus maintaining "normal BMI." Both conditions are correlated with higher morbidity and mortality (Summers et al. 2008; Giles et al. 2008; 2010). These conditions seem to be associated with the altered cytokine milieu in RA and especially with higher levels of TNF-α (also called cachectin) (Giles et al. 2010). Excess adipose tissue in RA is reported to have a predilection for accumulation in the visceral fatty beds, thus contributing to higher prevalence of central obesity especially in men, thus increasing the risk for cardiovascular disease (Giles et al. 2010). Beyond the loss in lean mass, there is evidence that muscle density is lower in RA than controls, presumably due to fatty infiltration, and possibly related to reduced number of contractile units and decreased strength (Kramer et al. 2012). While the research field of anthropometry and body composition alterations in RA was a largely uncharted territory until several years ago, it has attracted more attention recently and more light should be shed soon. Evidence suggests that TNF-α inhibition may reverse some of the effects of RA on body composition and resulting cachexia (Summers et al. 2008).

Pulmonary Involvement

Lung involvement is common in RA and may involve virtually every part of the respiratory apparatus including the upper and lower airways, the parenchyma, and the pleura. The prevalence of different forms of respiratory involvement is difficult to assess due to the frequency of subclinical disease, the variable sensitivity of detection methods, and the confounding influences of medications and infections. In this section, we will elaborate on the most common forms of respiratory involvement in RA that impact the most on morbidity and mortality.

Interstitial lung disease (ILD) is the most common and most serious form of parenchymal lung involvement in RA. It is reported to be the second highest cause of mortality in this patient population (Gabriel 2008). It may remain asymptomatic and detectable only with imaging or pulmonary function testing (PFT) for long periods of time, but once symptoms manifest, a progressive course usually ensues, leading to early mortality (Gabriel 2008). The lifetime risk for ILD has been reported to be more than 7% in subjects with RA versus less than 1% in the general population (Bongartz et al. 2010). Male patients who are older at the time of RA onset and with severe articular involvement are at higher risk (Anaya et al. 1995). The mortality associated with ILD in RA is nearly three times higher compared to the general population.

Once clinically manifested by cough and dyspnea, imaging generally demonstrates reticular, ground glass or honeycombing opacities, and PFT reveals restriction and a decrease in diffusion capacity.

Biopsy in RA-associated ILD – often necessary to differentiate between RA related ILD, infection, or medication toxicity – usually reveals patterns consistent with nonspecific or usual interstitial pneumonia (NSIP and UIP respectively), less commonly bronchiolitis obliterans organizing pneumonia (BOOP), and more rarely lymphocytic or desquamative interstitial pneumonia (LIP and DIP respectively) (Lee et al. 2005). Between NSIP and UIP, the former is considered to be more responsive to immune suppression with steroids (Flaherty et al. 2001).

BOOP is more dramatic in presentation with systemic features of inflammation (fatigue, fever, increased inflammatory markers) and, on imaging, has a characteristic appearance with patchy peripheral consolidations. It often has a less grave prognosis with favorable response to treatment with steroids.

The first line of therapy for this condition involves steroids in most of the times. In the absence of randomized clinical trials for the treatment of RA-related ILD, an inadequate response to steroids is usually followed by the addition of immunosuppressives such as azathioprine.

Pleural involvement is reported to be very common in RA often as an asymptomatic bystander. Small effusions or histologically detected pleural involvement may be present, but patients complain of symptoms rarely (Balbir-Gurman et al. 2006). It frequently occurs in patients with active

nodular disease and may be an incidental imaging finding or present with dyspnea.

Thoracentesis reveals an exudative effusion with typical characteristics being a low glucose level, a high LDH concentration, and the presence of giant multinucleated cells.

Therapy of the articular disease may alleviate pulmonary effusions or additional treatment with steroids or pleurodesis, and other invasive methods may be necessary.

Chronic obstructive pulmonary disease (COPD)/emphysema may also occur frequently in patients with RA. Difficulty in assessing its exact prevalence relates to the confounding effect of smoking and also to the fact that prevalent PFT abnormalities in patients with RA have been argued as being overall stable over time and not of significant clinical relevance (Hassan et al. 1994; Fuld et al. 2003).

Apart from the above, the respiratory system may be affected in several other ways. Upper airway involvement while frequently present with diagnostic methods only on occasion will cause symptoms such as hoarseness or odynophagia (Geterud et al. 1986). Laryngoscopy will assist in diagnosis and PFT may show characteristic changes in expiratory flow volumes. Obliterative bronchiolitis usually is accompanied by systemic manifestations and has a poor prognosis (Devouassoux et al. 2009). Bronchiectasis is frequently encountered with CT imaging (Remy-Jardin et al. 1994) but less commonly presents with symptoms. Rheumatoid nodules may be present in the lung parenchyma as mentioned earlier and when they are encountered in patients with pneumoconiosis constitute the Caplan syndrome which is only rarely recognized nowadays.

As alluded to earlier, lung involvement may be an extra-articular manifestation of RA but the differential diagnosis should always include infections, malignancies, and medication toxicity. Among the latter we will only mention here methotrexate-induced hypersensitivity acute pneumonitis, which usually has a rapid onset and is accompanied by fever and bilateral infiltrates in association with new and often severe respiratory symptoms (Dawson et al. 2002).

Cardiovascular Involvement

Cardiovascular involvement in RA is of particular importance, given the fact that it is recognized as the leading cause of mortality in this patient population (Myasoedova et al. 2010). Recently, research has demonstrated the RA-related inflammation might be an independent risk factor for the development of atherosclerotic disease, and subclinical diastolic dysfunction (Nicola et al. 2005; Arslan et al. 2006). Patients with RA are considered to be at increased risk for myocardial infarctions, strokes, and heart failure even after adjustment for smoking and other traditional cardiovascular risk factors (Crowson et al. 2005). Medications used to treat articular disease – such as TNF-α inhibitors – may mitigate this risk (Listing et al. 2008). Coronary vasculitis only rarely causes ischemic myocardial events in RA and usually only in patients with full-blown rheumatoid vasculitis and severe and active joint disease (Morris et al. 1986). Current guidelines advocate annual cardiovascular risk evaluation for patients with rheumatoid arthritis and tight control of articular disease activity and conventional cardiovascular risk factors (Peters et al. 2010).

Beyond the above, a plethora of other cardiac manifestations may be encountered in patients with RA, including pericarditis, myocarditis, and valvular disease as well as rheumatoid nodules interfering with valvular or conduction system function (Voskuyl 2006).

Pericardial disease is the most common cardiovascular manifestation. In most cases, it is clinically asymptomatic and only detected by echocardiography or in autopsy series with prevalence ranges estimated to more than 30 %. Clinically evident disease is usually seen in patients with active RA, and management of the articular disease may also improve the pericardial involvement (Voskuyl 2006).

Myocarditis apparently directly related to RA inflammation is increasingly recognized with MRI and PET imaging in a percentage of patients as a possible cause of heart failure (HF) in patients with RA (Voskuyl 2006). Two major pathologic patterns are encountered: either

a granulomatous pattern similar to rheumatoid nodules, or a nonspecific form (Lebowitz 1963). Both forms of RA myocarditis are considered putative causes for HF in RA.

The articular inflammation of RA when active and longstanding may lead to secondary (AA) amyloidosis (Husby 1985). Such a condition is rarely encountered today as RA arthritis medications can sufficiently render the articular inflammation into remission in the majority of patients. In the rare cases of myocardial involvement by amyloidosis, renal involvement is usually also present and a restrictive physiology may be seen on echocardiography with a characteristic sparkling pattern (Voskuyl 2006).

Patients with RA may have frequent cardiac atrial and ventricular arrhythmias. Cardiovascular autonomic dysfunction, defined by decreased heart rate variability and increased QT dispersion, also reportedly increased in RA, may also contribute to the increased risk of CV disease in RA (Stojanovich et al. 2007).

Other Organs' Involvement

As already mentioned, neurologic involvement in RA may be encountered in the context of rheumatoid vasculitis. However, a common non-vasculitic neurological complication of RA is peripheral nerve entrapment syndrome, most typically affecting the median nerve and manifesting as carpal tunnel syndrome. Local synovitis compressing the nerve is usually the culprit and treatment of synovitis may improve symptoms, reserving surgical intervention only for resistant cases.

Muscle weakness due to disuse atrophy is encountered in patients with RA usually with active disease that interfere with exercise and deconditioning. Cachexia and loss of muscle density and the possible association with decreased strength is mentioned in a different section in this entry. Although autopsy series have reported low grade inflammation in muscles of RA patients, clinically evident primary inflammation is rare. In the case of clinically profound weakness, the clinician should always consider medication toxicity such as hydroxychloroquine steroid or statin-induced myopathy.

Renal involvement is also rare in rheumatoid arthritis. Biopsies have shown mild mesangioproliferative glomerulonephritis in a small percentage of patients. Nephrotic syndrome is only rarely encountered nowadays, given the low incidence of secondary amyloidosis in RA. NSAID-related kidney toxicity is probably the most frequent renal manifestation in patients with RA.

Cross-References

- ▶ Rheumatoid Arthritis, Biologics in its Treatment
- ▶ Rheumatoid Arthritis, Clinical Features
- ▶ Rheumatoid Arthritis, Genetics
- ▶ Rheumatoid Arthritis, Treatment

References

Anaya JM, Diethelm L, Ortiz LA, Gutierrez M, Citera G, Welsh RA, et al. Pulmonary involvement in rheumatoid arthritis. Semin Arthritis Rheum. [Research Support, Non-U.S. Gov't Review]. 1995 Feb;24(4):242–54.

Arslan S, Bozkurt E, Sari RA, Erol MK. Diastolic function abnormalities in active rheumatoid arthritis evaluation by conventional Doppler and tissue Doppler: relation with duration of disease. Clin Rheumatol. 2006 May;25(3):294–9.

Balbir-Gurman A, Yigla M, Nahir AM, Braun-Moscovici Y. Rheumatoid pleural effusion. Semin Arthritis Rheum. [Review]. 2006 Jun;35(6):368–78.

Balint GP, Balint PV. Felty's syndrome. Best Pract Res Clin Rheumatol. [Research Support, Non-U.S. Gov't Review]. 2004 Oct;18(5):631–45.

Barber LD, Pflugfelder SC, Tauber J, Foulks GN. Phase III safety evaluation of cyclosporine 0.1 % ophthalmic emulsion administered twice daily to dry eye disease patients for up to 3 years. Ophthalmology. [Clinical Trial Clinical Trial, Phase III Multicenter Study Research Support, Non-U.S. Gov't]. 2005 Oct;112(10):1790–4.

Bartels C, Bell C, Rosenthal A, Shinki K, Bridges A. Decline in rheumatoid vasculitis prevalence among US veterans: a retrospective cross-sectional study. Arthritis Rheum. [Research Support, N.I.H., Extramural]. 2009 Sep;60(9):2553–7.

Bartels CM, Bell CL, Shinki K, Rosenthal A, Bridges AJ. Changing trends in serious extra-articular manifestations of rheumatoid arthritis among United State

veterans over 20 years. Rheumatology (Oxford). [Research Support, N.I.H., Extramural]. 2010 Sep;49(9):1670–5.

Bongartz T, Nannini C, Medina-Velasquez YF, Achenbach SJ, Crowson CS, Ryu JH, et al. Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study. Arthritis Rheum. [Research Support, N.I.H., Extramural]. 2010 Jun;62(6):1583–91.

Crowson CS, Nicola PJ, Kremers HM, O'Fallon WM, Therneau TM, Jacobsen SJ, et al. How much of the increased incidence of heart failure in rheumatoid arthritis is attributable to traditional cardiovascular risk factors and ischemic heart disease? Arthritis Rheum. [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S.]. 2005 Oct;52(10):3039–44.

Dawson JK, Graham DR, Desmond J, Fewins HE, Lynch MP. Investigation of the chronic pulmonary effects of low-dose oral methotrexate in patients with rheumatoid arthritis: a prospective study incorporating HRCT scanning and pulmonary function tests. Rheumatology (Oxford). [Clinical Trial Research Support, Non-U.S. Gov't]. 2002 Mar;41(3):262–7.

Devouassoux G, Cottin V, Liote H, Marchand E, Frachon I, Schuller A, et al. Characterisation of severe obliterative bronchiolitis in rheumatoid arthritis. Eur Respir J. 2009 May;33(5):1053–61.

Dinant HJ, Hissink Muller W, van den Berg-Loonen EM, Nijenhuis LE, Engelfriet CP. HLA-DRw4 in Felty's syndrome. Arthritis Rheum. [Letter]. 1980 Nov;23(11):1336.

Flaherty KR, Toews GB, Lynch JP, 3rd, Kazerooni EA, Gross BH, Strawderman RL, et al. Steroids in idiopathic pulmonary fibrosis: a prospective assessment of adverse reactions, response to therapy, and survival. Am J Med. [Research Support, U.S. Gov't, P.H.S.]. 2001 Mar;110(4):278–82.

Fujita M, Igarashi T, Kurai T, Sakane M, Yoshino S, Takahashi H. Correlation between dry eye and rheumatoid arthritis activity. Am J Ophthalmol. 2005 Nov;140(5):808–13.

Fuld JP, Johnson MK, Cotton MM, Carter R, Watkin SW, Capell HA, et al. A longitudinal study of lung function in nonsmoking patients with rheumatoid arthritis. Chest. 2003 Oct;124(4):1224–31.

Gabriel SE. Why do people with rheumatoid arthritis still die prematurely? Ann Rheum Dis. [Research Support, N.I.H., Extramural Review]. 2008 Dec;67 Suppl 3: iii30-4.

Geterud A, Ejnell H, Mansson I, Sandberg N, Bake B, Bjelle A. Severe airway obstruction caused by laryngeal rheumatoid arthritis. J Rheumatol. 1986 Oct;13(5):948–51.

Giles JT, Allison M, Blumenthal RS, Post W, Gelber AC, Petri M, et al. Abdominal adiposity in rheumatoid arthritis: association with cardiometabolic risk factors and disease characteristics. Arthritis Rheum. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2010 Nov;62(11):3173–82.

Giles JT, Ling SM, Ferrucci L, Bartlett SJ, Andersen RE, Towns M, et al. Abnormal body composition phenotypes in older rheumatoid arthritis patients: association with disease characteristics and pharmacotherapies. Arthritis Rheum. [Comparative Study Research Support, N.I.H., Extramural Research Support, N.I.H., Intramural Research Support, Non-U.S. Gov't]. 2008 Jun 15;59(6):807–15.

Goldberg J, Pinals RS. Felty syndrome. Semin Arthritis Rheum. [Case Reports]. 1980 Aug;10(1):52–65.

Guignard S, Gossec L, Bandinelli F, Dougados M. Comparison of the clinical characteristics of vasculitis occurring during anti-tumor necrosis factor treatment or not in rheumatoid arthritis patients. A systematic review of 2707 patients, 18 vasculitis. Clin Exp Rheumatol. [Comparative Study]. 2008 May-Jun;26(3 Suppl 49):S23-9.

Hassan WU, Keaney NP, Holland CD, Kelly CA. Bronchial reactivity and airflow obstruction in rheumatoid arthritis. Ann Rheum Dis. 1994 Aug;53(8): 511–4.

Husby G. Amyloidosis and rheumatoid arthritis. Clin Exp Rheumatol. [Research Support, Non-U.S. Gov't Review]. 1985 Apr-Jun;3(2):173–80.

Kaufmann J, Kielstein V, Kilian S, Stein G, Hein G. Relation between body mass index and radiological progression in patients with rheumatoid arthritis. J Rheumatol. 2003 Nov;30(11):2350–5.

Kerstens PJ, Boerbooms AM, Jeurissen ME, Fast JH, Assmann KJ, van de Putte LB. Accelerated nodulosis during low dose methotrexate therapy for rheumatoid arthritis. An analysis of ten cases. J Rheumatol. [Clinical Trial Randomized Controlled Trial]. 1992 Jun;19(6):867–71.

Kramer HR, Fontaine KR, Bathon JM, Giles JT. Muscle density in rheumatoid arthritis: associations with disease features and functional outcomes. Arthritis Rheum. 2012 Aug;64(8):2438–50.

Krishnaswamy G, Odem C, Chi DS, Kalbfleisch J, Baker N, Smith JK. Resolution of the neutropenia of Felty's syndrome by longterm administration of recombinant granulocyte colony stimulating factor. J Rheumatol. [Case Reports Review]. 1996 Apr;23(4):763–5.

Lamy T, Loughran TP, Jr. Clinical features of large granular lymphocyte leukemia. Semin Hematol. [Review]. 2003 Jul;40(3):185–95.

Lebowitz WB. The heart in rheumatoid arthritis (rheumatoid disease). A clinical and pathological study of sixty-two cases Ann Intern Med. 1963 Jan;58:102–23.

Lee HK, Kim DS, Yoo B, Seo JB, Rho JY, Colby TV, et al. Histopathologic pattern and clinical features of rheumatoid arthritis-associated interstitial lung disease. Chest. [Comparative Study]. 2005 Jun;127(6): 2019–27.

Listing J, Strangfeld A, Kekow J, Schneider M, Kapelle A, Wassenberg S, et al. Does tumor necrosis factor alpha

inhibition promote or prevent heart failure in patients with rheumatoid arthritis? Arthritis Rheum. [Research Support, Non-U.S. Gov't]. 2008 Mar;58(3):667–77.

Loughran TP, Jr. Clonal diseases of large granular lymphocytes. Blood. [Research Support, U.S. Gov't, P.H.S. Review]. 1993 Jul 1;82(1):1–14.

Morris PB, Imber MJ, Heinsimer JA, Hlatky MA, Reimer KA. Rheumatoid arthritis and coronary arteritis. Am J Cardiol. [Case Reports Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 1986 Mar 1;57(8):689–90.

Munro R, Capell H. Prevalence of low body mass in rheumatoid arthritis: association with the acute phase response. Ann Rheum Dis. 1997 May;56(5):326–9.

Myasoedova E, Davis JM, 3rd, Crowson CS, Gabriel SE. Epidemiology of rheumatoid arthritis: rheumatoid arthritis and mortality. Curr Rheumatol Rep. [Review]. 2010 Oct;12(5):379–85.

Narvaez J, Domingo-Domenech E, Gomez-Vaquero C, Lopez-Vives L, Estrada P, Aparicio M, et al. Biological agents in the management of Felty's syndrome: a systematic review. Semin Arthritis Rheum. [Case Reports Review]. 2012 Apr;41(5):658–68.

Nicola PJ, Maradit-Kremers H, Roger VL, Jacobsen SJ, Crowson CS, Ballman KV, et al. The risk of congestive heart failure in rheumatoid arthritis: a population-based study over 46 years. Arthritis Rheum. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2005 Feb;52(2):412–20.

Nyhall-Wahlin BM, Turesson C, Jacobsson LT, Nilsson JA, Forslind K, Albertsson K, et al. The presence of rheumatoid nodules at early rheumatoid arthritis diagnosis is a sign of extra-articular disease and predicts radiographic progression of joint destruction over 5 years. Scand J Rheumatol. [Research Support, Non-U.S. Gov't]. 2011 Mar;40(2):81–7.

Palmer DG, Hogg N, Highton J, Hessian PA, Denholm I. Macrophage migration and maturation within rheumatoid nodules. Arthritis Rheum. [Research Support, Non-U.S. Gov't]. 1987 Jul;30(7):728–36.

Patatanian E, Thompson DF. A review of methotrexate-induced accelerated nodulosis. Pharmacotherapy. [Review]. 2002 Sep;22(9):1157–62.

Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. Ann Rheum Dis. [Consensus Development Conference Practice Guideline Research Support, Non-U.S. Gov't]. 2010 Feb;69(2):325–31.

Puechal X, Miceli-Richard C, Mejjad O, Lafforgue P, Marcelli C, Solau-Gervais E, et al. Anti-tumour necrosis factor treatment in patients with refractory systemic vasculitis associated with rheumatoid arthritis. Ann Rheum Dis. 2008 Jun;67(6):880–4.

Ramos-Casals M, Brito-Zeron P, Munoz S, Soria N, Galiana D, Bertolaccini L, et al. Autoimmune diseases induced by TNF-targeted therapies: analysis of 233 cases. Medicine (Baltimore). 2007 Jul;86(4):242–51.

Rashba EJ, Rowe JM, Packman CH. Treatment of the neutropenia of Felty syndrome. Blood Rev. [Review]. 1996 Sep;10(3):177–84.

Remy-Jardin M, Remy J, Cortet B, Mauri F, Delcambre B. Lung changes in rheumatoid arthritis: CT findings. Radiology. 1994 Nov;193(2):375–82.

Rosenstein ED, Kramer N. Felty's and pseudo-Felty's syndromes. Semin Arthritis Rheum. [Review]. 1991 Dec;21(3):129–42.

Stojanovich L, Milovanovich B, de Luka SR, Popovich-Kuzmanovich D, Bisenich V, Djukanovich B, et al. Cardiovascular autonomic dysfunction in systemic lupus, rheumatoid arthritis, primary Sjogren syndrome and other autoimmune diseases. Lupus. 2007;16(3):181–5.

Summers GD, Deighton CM, Rennie MJ, Booth AH. Rheumatoid cachexia: a clinical perspective. Rheumatology (Oxford). [Review]. 2008 Aug;47(8):1124–31.

Suzuki A, Ohosone Y, Obana M, Mita S, Matsuoka Y, Irimajiri S, et al. Cause of death in 81 autopsied patients with rheumatoid arthritis. J Rheumatol. 1994 Jan;21(1):33–6.

Theodossiadis PG, Markomichelakis NN, Sfikakis PP. Tumor necrosis factor antagonists: preliminary evidence for an emerging approach in the treatment of ocular inflammation. Retina. [Review]. 2007 Apr-May;27(4):399–413.

Turesson C, Jacobsson LT. Epidemiology of extra-articular manifestations in rheumatoid arthritis. Scand J Rheumatol. [Comparative Study Review]. 2004;33(2):65–72.

Turesson C, McClelland RL, Christianson TJ, Matteson EL. No decrease over time in the incidence of vasculitis or other extraarticular manifestations in rheumatoid arthritis: results from a community-based study. Arthritis Rheum. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. 2004 Nov;50(11):3729–31.

Voskuyl AE, Zwinderman AH, Westedt ML, Vandenbroucke JP, Breedveld FC, Hazes JM. Factors associated with the development of vasculitis in rheumatoid arthritis: results of a case–control study. Ann Rheum Dis. [Comparative Study Research Support, Non-U.S. Gov't]. 1996 Mar;55(3):190–2.

Voskuyl AE. The heart and cardiovascular manifestations in rheumatoid arthritis. Rheumatology (Oxford). [Review]. 2006 Oct;45 Suppl 4:iv4-7.

Watson PG, Hayreh SS. Scleritis and episcleritis. Br J Ophthalmol. 1976 Mar;60(3):163–91.

Westhoff G, Rau R, Zink A. Radiographic joint damage in early rheumatoid arthritis is highly dependent on body mass index. Arthritis Rheum. [Research Support, Non-U.S. Gov't]. 2007 Nov;56(11):3575–82.

Wolfe F, Michaud K. Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. Arthritis Rheum. [Research Support, Non-U.S. Gov't]. 2004 Jun;50(6):1740–51.

Rheumatoid Arthritis, Genetics

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Definition

A discipline of science to clarify the contribution of genes in the etiology and pathophysiology of rheumatoid arthritis.

Genetic Factors in Rheumatoid Arthritis (RA)

RA is a systemic autoimmune disease that predominantly accompanies chronic synovial inflammation, which results in joint destruction and deformity. Approximately 1 % of the world population suffers from RA. It is widely accepted that both environmental and genetic factors contribute to the etiology of RA. Twin studies have revealed that genetic factors play a substantial role in the development of RA. For example, concordance rate for RA in monozygotic twins is four times larger than in dizygotic twins (MacGregor et al. 2000). Accordingly, overall contribution of genetic factors to RA is estimated to be 50–60 % (MacGregor et al. 2000; Seldin et al. 1999).

RA is heterogeneous in clinical manifestation and may have multiple causes (Aletaha et al. 2010; Arnett et al., 1988. Recent attempts to subclassify RA have shown that the presence of anti-citrullinated protein antibody (ACPA) has significant clinical relevance with the development of RA. ACPA is reasonably sensitive (68 %) and highly specific (98 %) for RA (Schellekens et al. 2000). ACPA is predictive for the development of RA: 75 % of patients with ACPA and undifferentiated arthritis would develop RA within 3 years (van Gaalen et al. 2004a). RA patients with ACPA exhibit more severe forms of the disease than those without ACPA (van Gaalen et al. 2004b). In addition, genetic risk profiles are different between ACPA-positive and -negative RA (D'Amato et al. 2010), suggesting differences in pathogenetic mechanisms between ACPA-positive and -negative RA. Recent genetic studies have analyzed not only clinically evident RA but also RA subtypes, for example, classified by the presence or absence of ACPA, as discussed below.

HLA (Human Leukocyte Antigen)-DRB1

Among multiple RA-susceptible alleles revealed so far, HLA-DRB1 risk alleles are most strongly associated with RA susceptibility (Plenge 2009; Raychaudhuri et al. 2010; Deighton et al. 1989). In the 1980s, it was discovered that multiple RA risk alleles in the HLA-DRB1 gene shared a common amino acid sequence, QKRAA, QRRAA, or RRRAA, which was designated "shared epitope" (SE) (Gregersen et al. 1987).

The SE is in the third hypervariable region of the DRB1 chain and situated in the α-helix of the peptide-binding groove (Gregersen et al. 1987). SE may therefore influence the species of peptides binding to the HLA molecule, hence the presentation of the peptides to T-cells; for example, arthritogenic peptides may be specifically bound to SE and interact with autoreactive T-cell receptors (TCRs) to activate arthritogenic CD (cluster of differentiation) 4^+ T-cells.

Notably, the association between SE allele and RA was only observed with ACPA-positive RA (Huizinga et al. 2005). Genetic risk factors associated with ACPA-negative RA appear to be much fewer than those associated with ACPA-positive RA partly because the cohorts analyzed so far mainly consist of ACPA-positive RA patients. Yet, one recent report showed that both ACPA-positive and ACPA-negative RA were equally heritable with approximately 65 % heritability for both diseases (van der Woude et al. 2009). However, only 2.4 % of the

heritability of ACPA-negative RA was due to SE, whereas 18 % of heritability of ACPA-positive RA was attributed to SE.

For ACPA-positive RA patients, smoking has been suggested as an important environmental risk factor, especially in those with one or two copies of the SE alleles (Kallberg et al. 2011; Linn-Rasker et al. 2006). In addition, enhanced expression of citrullinated proteins, as a result of induced expression of peptidylarginine deiminase-2, was found in the lungs of smokers (Makrygiannakis et al. 2008). It is thus plausible that smoking (an environmental factor) and SE (a genetic factor) cooperatively contribute to RA development. Certain autoantigens targeted in RA would be citrullinated as a result of smoking, thus acquiring a higher binding affinity for DRB1 molecule with SE; these citrullinated autoantigens displayed on SE are then recognized by autoreactive $CD4^+$ T-cells.

A recent genotype imputation approach has revealed variations of three amino acids at positions 11, 71, and 74 in HLA-DRB1 and single-amino-acid polymorphisms in HLA-B at position 9, which altogether could explain the HLA association with ACPA-positive RA (Raychaudhuri et al. 2012). The positions 71 and 74 are within an SE allele, and the position 11 is encoded by DNA (deoxyribonucleic acid) sequences approximately 200 base pairs away from those encoding the SE; all of these three amino acids are located within the peptide-binding groove of HLA-DRB1. Thus, peptides binding to the groove with SE may be presented for the activation of arthritogenic T-cells.

RA Risk Alleles Other Than HLA

Besides HLA, approximately 50 genes have been reported to have RA-associated risk alleles during last decade (Table 1), mainly as a result of the application of genome-wide association study (GWAS) for RA genetics. GWAS made it possible to conduct genetic studies of common variants across the entire human genome. Although exact causal mutations or genes are yet to be determined for most RA risk loci, it is of note that some RA risk alleles are common with other autoimmune diseases, and that there are substantial ethnic differences in RA risk alleles.

RA Risk Alleles Shared with Other Autoimmune Diseases

Many of RA risk loci (e.g., HLA-DR4, PTPN22 (Protein tyrosine phosphatase, non-receptor type 22), CTLA4 (Cytotoxic T-Lymphocyte Antigen 4), STAT4 (Signal Transducer and Activator of Transcription 4), IL2RA (interleukin 2 receptor alpha)) predispose to more than one autoimmune disease, including type 1 diabetes (T1D), celiac disease, systemic lupus erythematosus (SLE), inflammatory bowel disease, and multiple sclerosis. For example, PTPN22 is shared with T1D, SLE, and autoimmune thyroiditis (Begovich et al. 2004; Bottini et al. 2004; Criswell et al. 2005; Lee et al. 2007b; Orozco et al. 2005; Raychaudhuri et al. 2008). In most cases, the same allele is associated with these diseases. In addition, there are some gene loci that have several autoimmune alleles (e.g., TNFAIP3 (Tumor necrosis factor, alpha-induced protein 3) locus in RA, SLE, and T1D) (Fung et al. 2009; Graham et al. 2008; Musone et al. 2008; Plenge et al. 2007a; Thomson et al. 2007). The results suggest a common pathogenetic basis for autoimmune diseases.

Ethnic Differences

RA genetic studies so far conducted mainly on the populations of Caucasian and Asian populations have revealed clear ethnic differences in genetic predisposition to RA. For example, the peptidylarginine deiminase 4 (PADI4) gene is associated in several Asian populations in multiple studies (Ikari et al. 2005; Kang et al. 2006; Suzuki et al. 2003) and also in meta-analysis (Lee et al. 2007a). In contrast, it is not associated with RA susceptibility in Caucasians (Lee et al., 2007a; Plenge et al., 2005). Similarly, Fc receptor-like 3 (FCRL3)

Rheumatoid Arthritis, Genetics, Table 1 Non-HLA genes identified as RA susceptibility loci

Susceptibility gene	Variant	Published year	Authors
PADI4	rs2240340	2003	Suzuki, A., et al.
PTPN22	rs2476601, rs6679677	2004, 2007	Begovich, A,B., et al. WTCCC
FCRL3	rs7528684	2005	Kochi, Y., et al.
STAT4	rs7574865	2007	Remmers, E, F., et al.
TRAF1/C5	rs3761847	2007	Plenge, R, M., et al.
IRF5	rs3807306	2007	Sigurdsson, S., et al.
CTLA4	rs3087243, rs11571300, rs231735	2007, 2009	WTCCC, Gregersen, P, K., et al.
IL2RB	rs3218253, rs743777	2007, 2008	WTCCC, Barton, A., et al.
CD244	rs3766379	2008	Suzuki, A., et al.
CD40	rs4810485	2008	Raychaudhuri, S., et al.
CCL21	rs2812378	2008	Raychaudhuri, S., et al.
MMEL1/TNFRSF14	rs3890745	2008	Raychaudhuri, S., et al.
CDK6	rs42041	2008	Raychaudhuri, S., et al.
PRKCQ	rs4750316	2008	Raychaudhuri, S., et al.
KIF5A/PIP4K2C	rs1678542	2008	Raychaudhuri, S., et al.
TNFAIP3/OLIG3	rs6920220,	2008	Barton, A., et al.
AFF3	rs10865035	2009	Barton, A., et al.
IL2-IL21	rs6822844	2009	Barton, A., et al.
CD2/CD58	rs11586238	2009	Raychaudhuri, S., et al.
CD28	rs1980422	2009	Raychaudhuri, S., et al.
PRDM1	rs548234	2009	Raychaudhuri, S., et al.
REL	rs13031237	2009	Gregersen, P, K.., et al.
BLK	rs2736340	2009	Gregersen, P, K.., et al.
CCR6	rs3093024	2010	Kochi, Y., et al. Stahl, E, A., et al.
SPRED2	rs934734	2010	Stahl, E, A., et al.
ANKRD55/IL6ST	rs6859219	2010	Stahl, E, A., et al.
C5orf30	rs26232	2010	Stahl, E, A., et al.
PXK	rs13315591	2010	Stahl, E, A., et al.
RBPJ	rs874040	2010	Stahl, E, A., et al.
CCL21	rs951005	2010	Stahl, E, A., et al.
IL2RA	rs706778	2010	Stahl, E, A., et al.
MBP	rs2000811	2011	Terao, C., et al.
AIRE	rs2075876, rs760426	2011	Terao, C., et al.
SH2B3	rs653178	2011	Zhernakova, A., et al.
DDX6	rs10892279	2011	Zhernakova, A., et al.
CD247	rs864537	2011	Zhernakova, A., et al.
UBE3L3	rs2298428	2011	Zhernakova, A., et al.
UBASH3A	rs11203203	2011	Zhernakova, A., et al.
B3GNT2	rs11900673	2012	Okada, Y,. et al.
ANXA3	rs2867461	2012	Okada, Y,. et al.
CSF2	rs657075	2012	Okada, Y,. et al.
CD83	rs12529514	2012	Okada, Y,. et al.
NFKBIE	rs2233434	2012	Okada, Y,. et al.
ARID5B	rs10821944	2012	Okada, Y,. et al.
PDE2A/ARAP1	rs3781913	2012	Okada, Y,. et al.
PLD4	rs2841277	2012	Okada, Y,. et al.
PTPN2	rs2847297	2012	Okada, Y,. et al.

gene is associated with RA in Japanese populations (Ikari et al. 2006; Kochi et al. 2005), but not in Caucasian descents (Hu et al. 2006; Martinez et al. 2006). In contrast, PTPN22 (Begovich et al. 2004; Lee et al. 2007b; Plenge et al. 2005), TRAF (TNF receptor associated factors)/C (Complement) 5 (Kurreeman et al. 2007; Plenge et al. 2007b; Raychaudhuri et al. 2008), and TNFAIP3 (Plenge et al. 2007a; Raychaudhuri et al. 2008; Thomson et al. 2007) are susceptibility genes in European, but not Asian, populations. The reason for these ethnic differences is not clear and could be distinct with each gene.

Mechanisms of Rheumatoid Arthritis Implicated by Genetic Studies

Because the number of newly identified RA risk alleles is rapidly growing, it is difficult to draw an integrated picture of RA pathogenesis by simply taking all these risk alleles into consideration. Most of RA risk genes are related to immune function and could be involved in three possible pathways or steps for RA development:

1. T-cell activation by antigen presenting cells (MHC class II region, PTPN22, STAT4, CD28, CTLA4, CD83, UBASH3A (Ubiquitin-associated and SH3 domain-containing protein A), CD247, SH2B3 (SH2B adapter protein 3), CD2, and CD58)
2. CD40 signaling pathway and downstream activation of nuclear factor-KB (NF-KB) signaling pathway (CD40, TRAF1, TRAF6, TNFSF (Tumor necrosis factor superfamily) 14, TNFAIP3, REL, and NFKBIE (Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon))
3. IL-2 signaling pathway (IL2RA, IL2RB (Interleukin-2 receptor beta), and IL (Interleukin) 2/IL21)

Understanding how genetic variations in the function of these molecules contribute to RA development remains a challenge for future study.

Summary

To date, nearly 50 risk alleles have been described as associated with RA susceptibility. Among them, HLA-DRB1 risk alleles show the strongest association. Detailed analyses indicate that responsible polymorphisms are located within the peptide-binding grooves of HLA-DRB1 and possibly influence the display of self- or non-self-peptides that shape an arthritogenic TCR repertoire or activate arthritogenic T-cells. RA risk alleles other than HLA-DRB1 may affect immune regulation of the formation or activation of arthritogenic T-cells. The number of reported genetic risk alleles for RA is rapidly increasing mainly because of the application of GWAS. Hopefully, they will further elucidate the etiology and pathophysiology of RA.

Cross-References

- ▶ Autoantibodies in Rheumatoid Arthritis
- ▶ B7 and CD28 Families
- ▶ CD40
- ▶ CTLA4-Ig
- ▶ Environment and Autoimmunity
- ▶ NF-κB
- ▶ PTPN22
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham 3rd CO, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, et al. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against rheumatism collaborative initiative. Ann Rheum Dis. 2010;69:1580–8.

Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31:315–24.

Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, Ardlie KG,

Huang Q, Smith AM, Spoerke JM, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet. 2004;75:330–7.

Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, MacMurray J, Meloni GF, Lucarelli P, Pellecchia M, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet. 2004;36:337–8.

Criswell LA, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M, Moser KL, Begovich AB, Carlton VE, Li W, et al. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. Am J Hum Genet. 2005;76:561–71.

D'Amato M, Zucchelli M, Seddighzadeh M, Anedda F, Lindblad S, Kere J, Alfredsson L, Klareskog L, Padyukov L. Analysis of neuropeptide S receptor gene (NPSR1) polymorphism in rheumatoid arthritis. PLoS One. 2010;5:e9315.

Deighton CM, Walker DJ, Griffiths ID, Roberts DF. The contribution of HLA to rheumatoid arthritis. Clin Genet. 1989;36:178–82.

Fung EY, Smyth DJ, Howson JM, Cooper JD, Walker NM, Stevens H, Wicker LS, Todd JA. Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. Genes Immun. 2009;10:188–91.

Graham RR, Cotsapas C, Davies L, Hackett R, Lessard CJ, Leon JM, Burtt NP, Guiducci C, Parkin M, Gates C, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. Nat Genet. 2008;40:1059–61.

Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. 1987;30:1205–13.

Hu X, Chang M, Saiki RK, Cargill MA, Begovich AB, Ardlie KG, Criswell LA, Seldin MF, Amos CI, Gregersen PK, et al. The functional -169TC single-nucleotide polymorphism in FCRL3 is not associated with rheumatoid arthritis in white North Americans. Arthritis Rheum. 2006;54:1022–5.

Huizinga TW, Amos CI, van der Helm-van Mil AH, Chen W, van Gaalen FA, Jawaheer D, Schreuder GM, Wener M, Breedveld FC, Ahmad N, et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum. 2005;52:3433–8.

Ikari K, Kuwahara M, Nakamura T, Momohara S, Hara M, Yamanaka H, Tomatsu T, Kamatani N. Association between PADI4 and rheumatoid arthritis: a replication study. Arthritis Rheum. 2005;52:3054–7.

Ikari K, Momohara S, Nakamura T, Hara M, Yamanaka H, Tomatsu T, Kamatani N. Supportive evidence for a genetic association of the FCRL3 promoter polymorphism with rheumatoid arthritis. Ann Rheum Dis. 2006;65:671–3.

Kallberg H, Ding B, Padyukov L, Bengtsson C, Ronnelid J, Klareskog L, Alfredsson L. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. Ann Rheum Dis. 2011;70:508–11.

Kang CP, Lee HS, Ju H, Cho H, Kang C, Bae SC. A functional haplotype of the PADI4 gene associated with increased rheumatoid arthritis susceptibility in Koreans. Arthritis Rheum. 2006;54:90–6.

Kochi Y, Yamada R, Suzuki A, Harley JB, Shirasawa S, Sawada T, Bae SC, Tokuhiro S, Chang X, Sekine A, et al. A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. Nat Genet. 2005;37:478–85.

Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, van der Helm-van Mil AH, Allaart CF, Verduyn W, Houwing-Duistermaat J, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. PLoS Med. 2007;4:e278.

Lee YH, Rho YH, Choi SJ, Ji JD, Song GG. PADI4 polymorphisms and rheumatoid arthritis susceptibility: a meta-analysis. Rheumatol Int. 2007a;27:827–33.

Lee YH, Rho YH, Choi SJ, Ji JD, Song GG, Nath SK, Harley JB. The PTPN22 C1858T functional polymorphism and autoimmune diseases–a meta-analysis. Rheumatology (Oxford). 2007b;46:49–56.

Linn-Rasker SP, van der Helm-van Mil AH, van Gaalen FA, Kloppenburg M, de Vries RR, le Cessie S, Breedveld FC, Toes RE, Huizinga TW. Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles. Ann Rheum Dis. 2006;65:366–71.

MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, Silman AJ. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum. 2000;43:30–7.

Makrygiannakis D, Hermansson M, Ulfgren AK, Nicholas AP, Zendman AJ, Eklund A, Grunewald J, Skold CM, Klareskog L, Catrina AI. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. Ann Rheum Dis. 2008;67:1488–92.

Musone SL, Taylor KE, Lu TT, Nititham J, Ferreira RC, Ortmann W, Shifrin N, Petri MA, Kamboh MI, Manzi S, et al. Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus. Nat Genet. 2008;40:1062–4.

Orozco G, Sanchez E, Gonzalez-Gay MA, Lopez-Nevot MA, Torres B, Caliz R, Ortego-Centeno N, Jimenez-Alonso J, Pascual-Salcedo D, Balsa A, et al. Association of a functional single-nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with rheumatoid arthritis and systemic lupus erythematosus. Arthritis Rheum. 2005;52:219–24.

Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW, Wolfe F, Kastner DL, Alfredsson L, Altshuler D, et al. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. Am J Hum Genet. 2005;77:1044–60.

Plenge RM, Cotsapas C, Davies L, Price AL, de Bakker PI, Maller J, Pe'er I, Burtt NP, Blumenstiel B, DeFelice M, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet. 2007a;39:1477–82.

Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, Liew A, Khalili H, Chandrasekaran A, Davies LR, et al. TRAF1–C5 as a risk locus for rheumatoid arthritis–a genomewide study. N Engl J Med. 2007b;357:1199–209.

Plenge RM. Recent progress in rheumatoid arthritis genetics: one step towards improved patient care. Curr Opin Rheumatol. 2009;21:262–71.

Raychaudhuri S, Remmers EF, Lee AT, Hackett R, Guiducci C, Burtt NP, Gianniny L, Korman BD, Padyukov L, Kurreeman FA, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat Genet. 2008;40:1216–23.

Raychaudhuri S. Recent advances in the genetics of rheumatoid arthritis. Curr Opin Rheumatol. 2010;22:109–18.

Raychaudhuri S, Sandor C, Stahl EA, Freudenberg J, Lee HS, Jia X, Alfredsson L, Padyukov L, Klareskog L, Worthington J, et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. Nat Genet. 2012;44:291–6.

Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000;43:155–63.

Seldin MF, Amos CI, Ward R, Gregersen PK. The genetics revolution and the assault on rheumatoid arthritis. Arthritis Rheum. 1999;42:1071–9.

Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, Nagasaki M, Nakayama-Hamada M, Kawaida R, Ono M, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet. 2003;34:395–402.

Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, Donn R, Symmons D, Hider S, Bruce IN, et al. Rheumatoid arthritis association at 6q23. Nat Genet. 2007;39:1431–3.

van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL, Toes RE, Huizinga TW. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. Arthritis Rheum. 2004a;50:709–15.

van Gaalen FA, van Aken J, Huizinga TW, Schreuder GM, Breedveld FC, Zanelli E, van Venrooij WJ, Verweij CL, Toes RE, de Vries RR. Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. Arthritis Rheum. 2004b;50:2113–21.

van der Woude D, Houwing-Duistermaat JJ, Toes RE, Huizinga TW, Thomson W, Worthington J, van der Helm-van Mil AH, de Vries RR. Quantitative heritability of anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis. Arthritis Rheum. 2009;60:916–23.

Rheumatoid Arthritis, Treatment

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Synonyms

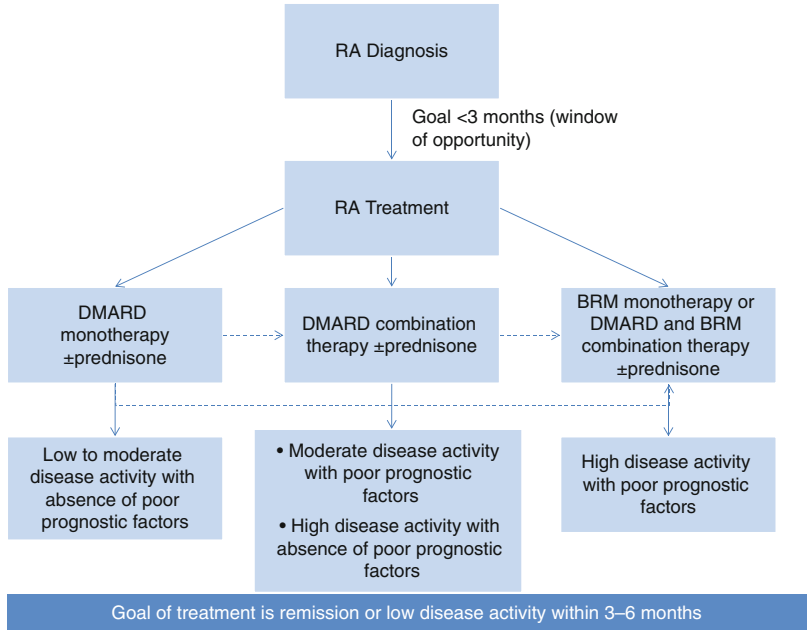
Biologic medications; Cytokines; Immunomodulators; Rheumatoid arthritis therapeutics; Treatment

Definition

Rheumatoid arthritis treatment pertains to the medical management of one of the most common autoimmune rheumatic diseases. Various therapeutic strategies exist and multiple different classes of medications have emerged and will be critically reviewed.

Introduction

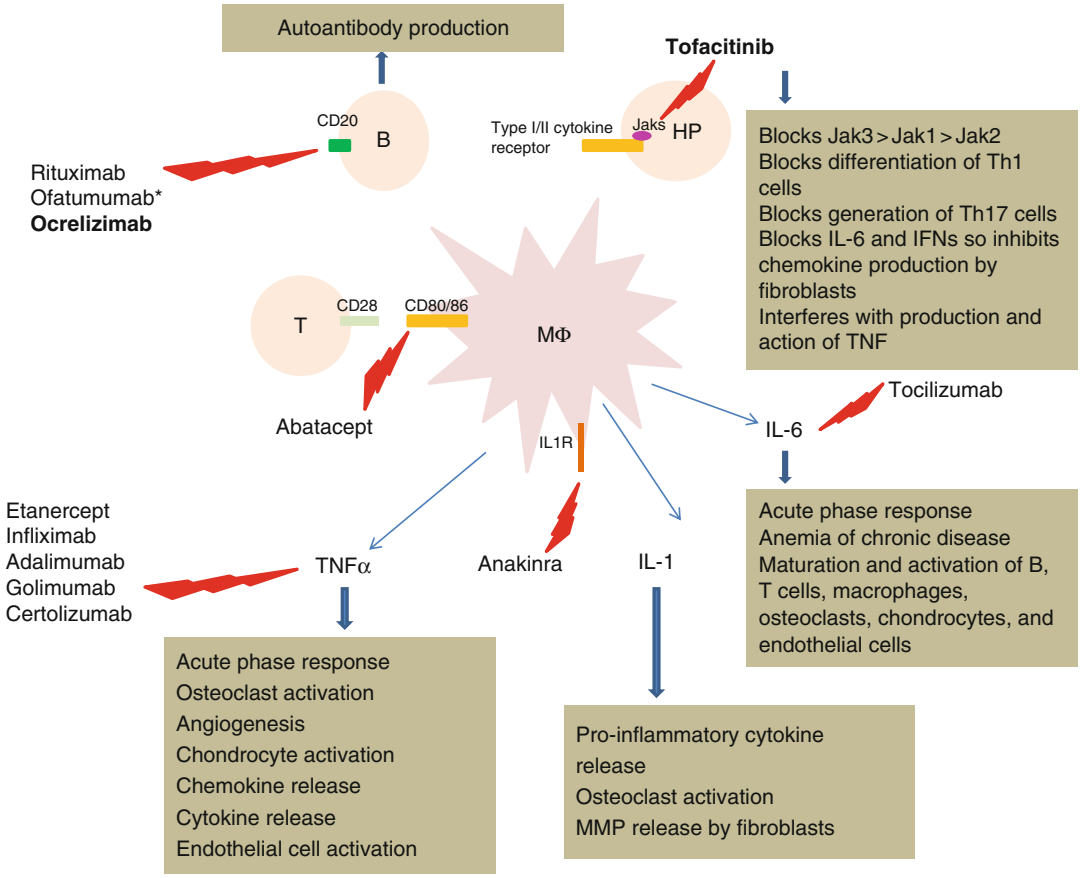
Since the mid-1990s, rheumatoid arthritis (RA) treatment has been revolutionized with the advent of molecular biology and the development of novel targeted biologic treatments, i.e., cytokine and cytokine receptor inhibitors. The therapeutic



Rheumatoid Arthritis, Treatment, Fig. 1 RA treatment algorithm. Ideally treatment should start within 3 months of diagnosis also known as window of opportunity. The goal is disease remission or minimal disease activity within 3–6 months form diagnosis. Different strategies exist depending on disease activity and patient prognostic factors. *BRM* Biologic Response Modifier, *DMARD* Disease-Modifying Antirheumatic Drug. **Disease activity:** Classified as low, moderate, and high, according to validated common scales such as Disease Activity Score in 28 joints (DAS-28) Remission: <2.6, Low activity: >2.6 to <3.2, Moderate activity: >3.2 to <5.1, High activity: >5.1. Other scales include the Patient Activity Scale (PAS) or PAS-II, Routine Assessment of Patient Index Data 3, Clinical Disease Activity Index, and the Simplified Disease Activity Index. **Poor prognostic factors:** extra-articular manifestations (rheumatoid nodules, Felty's syndrome, RA vasculitis), rheumatoid factor and/or anticyclic citrullinated peptide positivity, impaired functional ability (measured by Health Assessment Questionnaire (HAQ) or other validated tools), bone erosions by radiographs. *Dashed arrows* represent escalation of therapy in the absence of remission or significant improvement at 3-month re-assessment intervals

paradigm has been shifted from symptomatic relief toward earlier identification of disease and prompt initiation of disease-modifying treatment, aiming at structural preservation of the joints and improvement in function and quality of life. Traditional disease-modifying antirheumatic drugs (DMARDs) remain first-line agents in RA, with methotrexate (MTX) widely considered as the "anchor" drug. Other commonly prescribed DMARDs include leflunomide, sulfasalazine, and hydroxychloroquine. In an effort to achieve remission, current treatments target a variety of inflammatory pathways.

Combination regimens include multiple traditional DMARDs or combinations of a DMARD and a biologic response modifier (BRM). Different treatment strategies exist and, currently, sequential monotherapy and/or the step-up approach may be substituted with combination treatment, often including a BRM, in specific patient subsets with adverse prognostic factors (Fig. 1). Currently, different classes of BRMs exist, targeting a distinct niche of RA immunopathology, including cytokine inhibitors (TNF, IL-1, IL-6), B-cell-depleting agents (rituximab), T-cell co-stimulation inhibitors (abatacept), and small molecule tyrosine kinase (JAK, SYK) inhibitors in development (Fig. 2). However, even though modern RA treatment has drastically improved the natural course of the disease, achieving remission remains elusive in the majority of patients, underscoring the unmet need for further research that may lead to novel agents and/or strategies.



Rheumatoid Arthritis, Treatment, Fig. 2 Therapeutic targets in RA include cytokines such as TNF, IL-1, IL-6, B cells, T-cell co-stimulation, and kinases. Cytokines exert pleiotropic effects, predicting the beneficial action of cytokine blockers. Tofacitinib and fostamatinib targeting JAK3/JAK1 and SYK kinases exert their beneficial actions through different mechanisms shown above

Traditional Disease-Modifying Antirheumatic Drugs (DMARDs)

Corticosteroids and nonsteroid anti-inflammatory agents (NSAIDs) were the mainstay of RA treatment before the institution of current DMARD therapy, which is proven to delay disease progression by reducing joint destruction. Even though corticosteroids are shown to impede radiographic progression (Kirwan 1995), they are currently used at the lowest possible dose for short-term periods, as adjunct agents for symptomatic relief, in view of their well-established long-term side-effect profile including osteoporosis, impaired glucose tolerance, cataract induction, and hypertension.

The most commonly recommended DMARDs include MTX, hydroxychloroquine, sulfasalazine, and leflunomide. Historically, other DMARDs have been used, such as azathioprine, penicillamine, gold salts, minocycline, doxycycline, and cyclosporine; however, their use has been largely abandoned due to increased toxicity and/or lack of efficacy. DMARD choice depends on patients' prognostic features, comorbidities, and preferences, individual tolerance to the respective medication and the drug safety profile. Concurrently, adverse prognostic factors such as presence of radiographic progression (erosions, joint space narrowing), presence of the shared epitope, presence of anti-CCP antibodies, and rheumatoid factor could dictate an earlier and

more aggressive approach, often requiring combination therapy with DMARDs (usually MTX) and a BRM (Markatseli et al. 2010).

Methotrexate

MTX is a potent anti-inflammatory and antiproliferative agent. It exerts its effects by virtue of increased levels of adenosine and through inhibition of transmethylation reactions. MTX is a folate analogue, which rapidly enters dividing cells though a folate receptor. Intracellularly, MTX is polyglutamated interfering with enzymes ultimately leading to increased levels of adenosine. Adenosine has potent anti-inflammatory properties and it is shown to inhibit the pro-inflammatory action of many key cytokines (Canella and O'Dell 2008).

MTX is the cornerstone of DMARD therapy in RA and is indicated for patients who have moderate to severe disease and radiologic evidence of joint erosions. Once MTX efficacy and safety was established as monotherapy, it was quickly realized that its combination with other traditional DMARDs such as hydroxychloroquine and sulfasalazine (triple therapy) could be even more effective (Coury and Weinblatt 2010). Initial combination therapy as a therapeutic strategy was formally tested within the BeST trial, where 4 different approaches were compared: monotherapy, step-up combination with traditional DMARDs, step-down therapy or initial combination therapy with MTX and infliximab, a TNF-α inhibitor. Although, all strategies showed clinical efficacy at 1 year, combination therapies led to earlier clinical remission and less structural damage (Goekoop-Ruiterman et al. 2005). In a series of randomized control trials in MTX-naive patients with early RA (onset of symptoms within 3 years), the combination of MTX and an anti-TNF-α agent (either infliximab, etanercept or adalimumab) or abatacept, a T-cell co-stimulation blocker, was superior to MTX alone in terms of disease activity, radiographic progression, and functional outcomes (Coury and Weinblatt 2010). MTX has also been efficacious in combination with rituximab, a peripheral B-cell depletor, at the dose of 1000 mg, given twice, at a 2-week interval.

MTX can be administered either orally or subcutaneously at initially low doses of 7.5 mg to 10 mg weekly escalating to 20–25 mg weekly. Concurrent folic acid administration at a dose of 1–3 mg daily may decrease the incidence of gastrointestinal and hematologic side effects stemming from its mechanism of action.

Side effects frequently result from folate depletion and may be reversible with folic acid replacement. Nausea, dyspepsia, and oral ulcers are common. Elevation of liver function tests is dose dependent and can be more severe in patients with HBV or HCV and in patients consuming excessive alcohol (which are situations in which methotrexate is contraindicated). Cytopenias secondary to bone marrow toxicity are relatively rare and caution should be exercised in patients with impaired renal function because MTX metabolites are renally excreted. Folinic acid (leucovorin) administration can be given as rescue in cases of severe bone marrow toxicity. Pulmonary toxicity can present as hypersensitivity pneumonitis necessitating discontinuation of MTX and administration of systemic corticosteroids once other causes (e.g., infections) are ruled out. Interstitial lung disease has been rarely reported in cases where high cumulative doses are given long term. Associations of MTX with malignancies especially lymphoma have been reported, but no causative link has been established in RA, given the increased risk of lymphoma in RA, even in untreated patients (Wolfe and Michaud 2004).

Hydroxychloroquine

Hydroxychloroquine is an antimalarial agent with anti-inflammatory and immunomodulatory properties. As a lipophilic weak base, it enters lysosomes raising pH and therefore interfering with protein processing. Consequently, antigen presentation as well as cell-mediated cytotoxicity is impaired. Decrease of auto-reactive lymphocytes is reported as a result of upregulated apoptosis. More recently, hydroxychloroquine has been found to inhibit intracellular Toll-like receptors such as TLR-9, modulating innate immune responses and antigen presentation (Katz and Russell 2011).

Hydroxychloroquine has demonstrated modest efficacy in RA and it is typically reserved for

mild disease with few or no adverse prognostic factors and as part of combination treatment, such as triple therapy together with sulfasalazine and methotrexate (Gaujoux-Viala et al. 2010).

Hydroxychloroquine is administered orally at a maximum dose of 6.5 mg/kg of ideal body weight and has a large distribution volume. Its long half-life renders hydroxychloroquine a slow-acting drug. It has a favorable safety profile, including in pregnant and lactating women, with retinal toxicity being the most serious complication, albeit rare and usually reversible upon discontinuation. Due to the agent's accumulation in melanin-storing cells, skin can also be affected, causing rash and hair depigmentation. Other common side effects are nausea, vomiting, diarrhea, and hypoglycemia, especially in diabetic patients already on glucose-lowering medications and tight diabetes control. Rarely, neuromuscular toxicity is reported manifesting with indolent proximal weakness and normal creatine kinase.

Sulfasalazine

Sulfasalazine consists of two moieties: 5-aminosalicylic acid (5-ASA) possessing anti-inflammatory properties and the antibacterial sulfapyridine. Although sulfasalazine has been one of the first drugs to be specifically employed for RA, its full mechanism of action in vivo is yet to be understood. It is suggested to inhibit arachidonic acid cascade and increase adenosine levels by inhibiting intracellular enzymes similarly to MTX (Canella and O'Dell 2008).

Its use in RA is typically restricted to those with mild disease, as an alternative to MTX where contraindicated or combined with hydroxychloroquine and MTX. It is also considered relatively safe along with hydroxychloroquine for women contemplating pregnancy. It is administered orally initially at doses 500 mg daily escalating up to 1,500–3,000 mg daily as tolerated.

The most common side effects are nausea, epigastric discomfort, headache, and dizziness. Hematologic abnormalities usually occur within the first 3 months of therapy and may include leukopenia, rarely agranulocytosis, and hemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency. Photosensitive rash has been reported and patients should be cautioned against taking sulfonamide-containing drugs (Canella and O'Dell 2008).

Leflunomide

Leflunomide is a prodrug which is rapidly converted to its active metabolite in the liver. It is considered to be a T-cell immunomodulator by inhibiting pyrimidine synthesis via reversible inhibition of the enzyme dihydroorotate dehydrogenase. Moreover, it acts as a tyrosine kinase inhibitor, interfering with signal transduction which is critical in immune cell growth and metabolism (Canella and O'Dell 2008).

Leflunomide has a 2-week half-life and it may be detected long after it has been discontinued. Given that it is teratogenic, patients contemplating pregnancy should have levels of its active metabolite measured and if higher than 0.02 mg/L, an elimination procedure with cholestyramine (2 g three times daily for 8 days) is recommended and levels rechecked for acceptable levels at least twice, in 2-week intervals (Canella and O'Dell 2008).

Leflunomide is an effective agent with onset of response seen within 4–12 weeks. A loading dose of 100 mg daily for 3 days is followed by a maintenance dose of 10–20 mg per day. Some expert clinicians advocate against the loading dose, in order to prevent adverse effects leading to attrition such as diarrhea, nausea, and abnormal liver function tests. Leflunomide is shown to retard radiographic progression and is considered to be a second-line agent in patients who are intolerant or failed MTX. Experience with leflunomide as a combination therapy is not as extensive as with MTX and frequent monitoring for toxicity is warranted (Maddison, Kiely et al. 2005). Side effects also include hypertension, hypercholesterolemia, weight loss, rash, interstitial lung disease, and, rarely, pancytopenia (Canella and O'Dell 2008).

Biological Response Modifiers (BRMs)

Biologic response modifiers have revolutionized the practice of rheumatology by improving

clinical outcomes and quality of life, arresting disease progression and preventing disability in a significant percentage of patients. The first class of BRMs that entered the clinic was the TNF-α inhibitors. Subsequently, advances in our understanding of RA immunopathophysiology allowed for additional target identification, including cytokines (IL-1, IL-6), B- and T-cells as well as signal transduction molecules.

Anti-TNFs

TNF-α has an established pivotal role as a driving cytokine in RA pathogenesis, mediating symptoms, and structural articular damage. TNF-α inhibitors have pleiotropic effects including complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, reverse signaling, and cytokine suppression (Taylor 2010).

Currently, there are five anti-TNF drugs approved for moderate to severe RA resistant to DMARDs, such as MTX. Despite variable pharmacokinetic properties and distinctive differences in design, they have all shown similar efficacy in randomized controlled clinical trials. Given our significant experience with the entire class of anti-TNF agents in terms of efficacy and safety, the bar has been raised for novel candidate agents to be tested in RA. Anti-TNF therapies have been associated with rare, albeit serious and occasionally fatal complications, including the elevated infection risk (especially opportunistic infections such as tuberculosis), malignancies, heart failure, demyelinating disorders, and reactivation of hepatitis B (Aaltonen et al. 2012). Concurrent administration of MTX, even at low doses, has been shown to decrease the incidence of immunogenicity and production of auto-antibodies against these agents.

Infliximab

Infliximab is a chimeric (70 % human 30 % mouse) IgG1 monoclonal antibody that binds to cell-bound and circulating TNF-alpha with high affinity. Its half-life is 8–10 days. It is administered intravenously (iv) at a dose of 3 mg/kg, followed by repeat dosing at 2 and 6 weeks (loading) and thereafter every 8 weeks (maintenance). Unresponsive patients might benefit from increasing the dose (up to 10 mg/kg) or by increasing the infusion frequency to a maximal frequency of every 4 weeks.

Infliximab's efficacy in RA has been demonstrated in combination with MTX in multiple double-blind, randomized, placebo-controlled trials, mostly in patients who had failed DMARDs (including MTX) (Aaltonen et al. 2012) (Table 1). Infliximab monotherapy was only initially tested at small open-label trials to demonstrate efficacy and safety. However, given the emerging data that combination with MTX may be clinically superior and may also decrease the risk of immunogenicity, all subsequent studies tested the combination of infliximab with MTX versus MTX alone (placebo). Infliximab has been tested both in long-standing disease and early RA (3 years or less of duration). Interestingly, in early RA, joint destruction was averted even in the absence of clinical improvement in a subset of patients, suggesting that there might be uncoupling of radiographic disease progression and clinical response (signs and symptoms).

Etanercept

Etanercept is a humanized, fusion protein generated by the linkage of two ligand binding regions of p75 TNF-α receptor and the Fc portion of human IgG1, and possesses the shortest half-life (3–4 days) of all the TNF-α inhibitors. Unlike other TNF-inhibitors, it binds both TNF-α and TNF-β.

Etanercept is administered subcutaneously (SC) weekly at a dose of 50 mg or twice weekly at a dose of 25 mg. It is approved both as monotherapy and in combination with MTX in moderate to severe RA (Table 1). Etanercept was the first anti-TNF to be tested as monotherapy in double-blind placebo-controlled randomized trials (Aaltonen et al. 2012). TEMPO trial was the first to demonstrate that the "add-on" trial design, at least in the case of etanercept, is not

Rheumatoid Arthritis, Treatment, Table 1 Pivotal clinical trials of TNF-alpha inhibitors in rheumatoid arthritis

TNF-alpha inhibitors						
Agents	Clinical trial	# Pts.	Study length	Intervention	Prior DMARD use	Significant outcome measures ($p \leq 0.05$)
Infliximab	ATTRACT, Maini	428	30 weeks	MTX + PLB vs MTX + IFX	MTX-IR	ACR20, ACR50
	St Clair et al.	1049	54 weeks	MTX + PLB vs MTX + 3 mg/kg IFL vs MTX + 6 mg/kg IFL	MTX-Naïve	ACR20/50/70, VdH-S
	ASPIRE, Smolen	1004	54 weeks	IFX + MTX vs PLB + MTX	MTX-Naïve	SHS
	BeSt, Goekoop-Ruiterman et al.	508	24 months	DMARD sequential monotherapy vs DMARD combination step up vs DMARD combination + prednisone vs DMARD + IFL	MTX-Naive	DAS, SHS
	Quinn et al.	20	12 months	IFX + MTX vs MTX + PLB	MTX-Naïve	ACR20/50/70, DAS28, MRI evidence of synovitis
Etanercept	Moreland et al.	180	3 months	ETN vs PLB	DMARDs-IR	ACR20/50
	Moreland et al.	234	6 months	ETN vs PLB	DMARDs-IR	ACR20/50
	COMET, Emery	542	52 weeks	MTX vs MTX + ETN	MTX-Naïve	DAS28, SHS
	TEMPO, van der Heijde, D.	503	24 months	MTX vs ETN vs MTX + ETN	DMARDs-IR	ACR20/50/70, DAS28, VdH-S
Adalimumab	PREMIER, Breedveld et al.	799	24 months	MTX vs ADA vs MTX + ADA	MTX-Naïve	ACR50, VdH-S, DAS28
	Chen et al.	47	12 weeks	MTX + PLB vs MTX + ADA	MTX-IR	ACR20/50/70
	ARMADA, Weinblatt et al.	271	4 years	MTX + PLB vs MTX + ADA	MTX-IR	ACR20/50/70, DAS28
Certolizumab	Smolen et al.	619	24 weeks	MTX + PLB vs MTX + CRT	MTX-IR	ACR20, VdH-S, HAQ-DI
	Keystone et al.	982	52 weeks	MTX + PLB vs MTX + CRT	MTX-IR	ACR20, VdH-S
	FAST4WARD, Fleischmann	220	24 weeks	CRT vs PLB	DMARDs-IR	ACR20/50/70, DAS28, HAQ-DI, VAS
Golimumab	GO-FORWARD, Keystone et al.	633	24 weeks	MTX + PLB vs GLB + PLB vs GLB + MTX	MTX-IR	ACR20/50/70, DAS28, HAQ-DI
	Kay et al.	172	52 weeks	MTX + PLB vs GLB + MTX	MTX-IR	ACR20, DAS28
	GO-AFTER, Smolen et al.	461	24 weeks	GLB + DMARD vs PLB + DMARD	TNF-IR	ACR20, HAQ
	GO-BEFORE, Emery et al.	637	24 weeks	MTX + PLB vs GLB + PLB vs GLB + MTX	MTX-Naïve	ACR50 (primary end point not achieved), DAS28
	GO-FORTH, Tanaka et al.	269	14 weeks	MTX + PLB vs GLB 50 mg + MTX vs GLB 100 mg + MTX	MTX-IR	ACR20

reflective of pharmacokinetic interaction of the study agent and MTX but rather an effect that may be dependent on the agents' mechanism of action.

Adalimumab

Adalimumab is a recombinant human IgG1 monoclonal antibody against TNF-α with an estimated half-life of 6–14 days, which can be used as monotherapy or in combination with MTX for the treatment of RA. It is administered subcutaneously at a dose of 40 mg every 2 weeks and is often titrated up to weekly, if necessary (approved in this higher dosage if taken without MTX). As with etanercept, adalimumab was tested in MTX non-responders (ARMADA study) and in MTX naive patients with early RA (PREMIER study, disease duration less than 3 years). The combination with MTX was clinically superior, with less radiographic progression at years 1 and 2 compared to monotherapy with either MTX or adalimumab. Adalimumab monotherapy compared to MTX monotherapy was also effective in arresting radiographic outcomes with comparable clinical efficacy (Aaltonen et al. 2012) (Table 1).

Golimumab

Golimumab is a humanized TNFα blocker binding to both soluble and transmembrane forms of TNF-α given every 4 weeks by a subcutaneous injection at a dose of 50 mg. Efficacy of golimumab with MTX has been demonstrated in RA patients who were MTX-naive, MTX-inadequate responders, and, for the first time in a randomized controlled study, TNF-inhibitor-inadequate responders, supporting the current practice pattern of "cycling" different TNF-blockers in patients who have an inadequate response to one TNF-inhibitor (Boyce et al. 2010) (Table 1).

Certolizumab

Certolizumab pegol is the pegylated portion of the Fab' fragment derived from a TNF-α monoclonal antibody, given subcutaneously once every two weeks. It is the only sc TNF-inhibitor with a loading dose (400 mg at weeks 0, 2, 4) and, for maintenance, it can be administered either every 2 weeks (200 mg) or every 4 weeks (400 mg). The absence of Fc region renders the drug incapable of inducing complement- or antibody-dependent cell-mediated cytotoxicity, differentiating certolizumab from the other anti-TNFs. Furthermore, the lack of the Fc portion may translate in hindered active transfer through the placenta in pregnant women. Clinical trials have proven certolizumab to be efficacious, both as monotherapy and as an add-on therapy to MTX improving the signs and symptoms of RA as early as week 1 and inhibiting the progression of structural joint damage as early as 16 weeks (Mease 2011) (Table 1).

Interleukin-1 Blockade

Interleukin-1 belongs to the cytokine milieu known to drive the pathophysiology of RA. This is established in various preclinical RA models and in vitro where both IL-1α and IL1β are proven to induce cytokine production by synovial mononuclear cells and chondrocytes and also drive bone erosion following osteoclast activation.

Anakinra

Anakinra is the recombinant form of human IL-1 receptor antagonist (IL-1ra) that is currently approved by the US Food and Drug Administration (FDA) for moderate to severe RA that has been unresponsive to initial DMARD therapy. In Europe, this agent has been approved for the treatment of RA only in combination with MTX whereas in USA, monotherapy may also be instituted (Mertens and Singh 2009) (Table 2).

It is administered as a daily subcutaneous injection at a dose of 100 mg and adverse effects primarily include injection-site reactions and increased rate of serious infections, yet not statistically significant in randomized trials compared to placebo (Mertens and Singh 2009).

The fact that the absolute treatment benefit with anakinra does not reach that of anti-TNFs and the need for daily administration has led to its infrequent use in adults with RA. Longer acting IL-1 inhibitors, such as rilonacept and canakinumab, primarily used for

Rheumatoid Arthritis, Treatment, Table 2 Pivotal clinical trials of IL-1 inhibitors in rheumatoid arthritis

IL-1 inhibitors						
Agents	Clinical trial	# Pts.	Study length	Intervention	Prior DMARD use	Significant outcome measures ($p \leq 0.05$)
Anakinra	Karanikolas et al.	128	48 weeks	DMARD vs DMARD + ANA	DMARDs-IR	ACR20/50/70, DAS28
	Cohen et al.	506	24 weeks	MTX + PLB vs MTX + ANA	MTX-IR	ACR20/50/70
	Cohen et al.	419	24 weeks	MTX + PLB vs MTX + ANA (6 dosing regimens)	MTX-IR	ACR20
	Jiang et al.	472	48 weeks	ANA 30 mg vs ANA 75 mg vs ANA 150 mg vs PLB	MTX-Naïve	GS, LS
	Bresnihan et al.	472	76 weeks	ANA 30 mg vs ANA 75 mg vs ANA 150 mg vs PLB	MTX-Naïve	ACR20/50/70

auto-inflammatory syndromes, have not been studied in RA (Mertens and Singh 2009).

Interleukin-6 Blockade

Interleukin-6 is known to play a critical role in RA pathogenesis, as evidenced by increased levels both in serum and synovial tissue which also significantly correlate with disease activity reflected in joint destruction and inflammatory markers. Preclinical models of IL-6 knockout mice were protected by collagen-induced arthritis laying the foundations for subsequent clinical development of an IL-6 inhibitor for RA.

Tocilizumab

Tocilizumab is a humanized IgG1 monoclonal antibody against the soluble and membrane-bound 80 kD component of the IL6 receptor, thereby inhibiting the homodimerization of gp130 and downstream signal transduction.

Tocilizumab has been approved for the treatment of RA in the USA after failure of at least one TNF-inhibitor, at a dose of 4 or 8 mg/kg given intravenously every 4 weeks, based on its efficacy in patients who had previously failed to respond to DMARDs or anti-TNF agents or both (Singh et al. 2011). Weekly or biweekly subcutaneous injections are being tested in early phase clinical trials. Overall, combination therapy of tocilizumab and MTX has resulted in superior efficacy than MTX alone with side effects being infusion-related reaction, increased frequency of infections, cytopenias, hyperlipidemia, and hypertension (Singh et al. 2011) (Table 3).

B-Cell-Depleting Agents

The presence of auto-reactive B cells resulting in the production of auto-antibodies highlights the key role of B-cell compartment in RA pathogenesis. It has also been convincingly shown that Bcells may also function as antigen-presenting cells, especially at the tissue level, and as cytokine secretors, driving the inflammatory process. Expression of CD20 on distinct stages of B-cell development was initially utilized to target peripheral B-cells in patients with non-Hodgkin lymphoma and the strategy was, more recently, tried in RA.

Rituximab

Rituximab is a chimeric human/mouse anti-CD20 antibody administered intravenously in two doses of 1000 mg, 2 weeks apart. Rituximab leads to apoptosis via antibody-dependent cytotoxicity and/or complement-dependent cytotoxicity. Pre-medication with steroids is indicated, especially prior to the first dose, to decrease the frequency and severity of infusion reactions. Rituximab induces a rapid depletion of CD20-expressing B-cells in the peripheral

Rheumatoid Arthritis, Treatment, Table 3 Pivotal clinical trials of IL-6 receptor inhibitors in rheumatoid arthritis

IL-6 receptor inhibitors						
Agents	Clinical trial	# Pts.	Study length	Intervention	Prior DMARD use	Significant outcome measures ($p \leq 0.05$)
Tocilizumab	AMBITION, Jones et al.	673	24 weeks	TCZ + MTX vs MTX + PLB	MTX-Naïve	ACR20, DAS28
	CHARISMA, Maini et al.	359	20 weeks	TCZ vs TCZ + MTX vs MTX + PLB	MTX-IR	ACR20/50/70, DAS28
	SATORI, Nishimoto et al.	125	24 weeks	TCZ + PLB vs MTX + PLB	MTX-IR	ACR20, DAS28
	SAMURAI, Nishimoto et al.	306	52 weeks	TCZ vs DMARDs	DMARD-IR	Mean total modified Sharp score (TSS)
	ROSE, Yazici et al.	614	24 weeks	TCZ + DMARD vs PLB + DMARD	DMARD_IR	ACR50, ACR 20/70, EULAR response, DAS28

blood, and levels remain low or undetectable for 2–6 months before returning to pretreatment levels at a mean of 8 months. Of note, hypogammaglobulinemia has not been consistently reported. In the USA, rituximab use is reserved for incomplete responders to TNF-inhibitors. It appears that clinical efficacy may be predicted by rheumatoid factor, albeit not anti-CCP, positivity (Benucci et al. 2010) (Table 4).

New-Generation Anti-CD20 Monoclonal Antibodies

In an effort to minimize infusion reactions, presumably due to the chimeric nature of rituximab, second-generation humanized anti-CD20 agents were developed. They also have the theoretical advantage of not triggering human anti-chimeric antibodies resulting in loss of efficacy similarly to anti-TNFs, although not yet reported with rituximab in RA. Ocrelizimab, which binds to a different epitope of CD20, was bedeviled in clinical trials by serious and opportunistic infections, especially at the higher doses where efficacy was seen, leading to suspension of phase III trials for RA. Ofatumumab, another humanized anti-CD20 monoclonal antibody, again binding to a different epitope, showed promise in phase I/II trials with no increase in safety signals (Buch and Emery 2011).

T-Cell c-Stimulation Blockade

T-cell activation is crucial in the induction of adaptive immunity in RA. Classically, T-cells are activated in a stepwise fashion during their interaction with the antigen-presenting cells (APCs). The first signal consists of presentation of the antigen loaded on major histocompatibility complex molecule to the T-cell receptor. Full T-cell activation requires a second co-stimulatory signal mediated by the interaction of APC surface molecules (CD80/CD86) and CD28 on T-cells. Although multiple co-stimulation mechanisms have been described, the CD80/86-CD28 pathway remains the best characterized.

Abatacept

Abatacept (CTLA4-Ig), a selective T-cell co-stimulation modulator, is administered as an intravenous infusion at week 0, 2, week 4, and every 4 weeks thereafter with the dose determined by body weight (500–1000 mg). It is also available on subcutaneous formulation with similar efficacy and safety profile with no evidence of immunogenicity. The subcutaneous injection is administered as a fixed dose (125 mg weekly) with or without an initial, weight-based, intravenous loading dose. Mechanistically, it binds to CD80 and CD86 blocking the co-stimulatory signal and optimal T-cell activation. This strategy has been employed for the treatment of solid tumors and hematological malignancies (Pham et al. 2012).

In RA, it is indicated as monotherapy or in combination with DMARDs (but not with other biologics) based on clinical and radiographic superior outcome compared to placebo in both

Rheumatoid Arthritis, Treatment, Table 4 Pivotal clinical trials of B-cell depleting agents in rheumatoid arthritis

B-cell depleting agents						
Agents	Clinical trial	# Pts.	Study length	Intervention	Prior DMARD use	Significant outcome measures ($p \leq 0.05$)
Rituximab	Edwards et al.	161	48 weeks	MTX vs RTX vs RTX + CYC vs RTX + MTX	MTX-IR	ACR50, ACR 20/70, EULAR response, DAS28
	DANCER, Emery et al.	465	24 weeks	MTX + PLB vs MTX + RTX 500 mg vs MTX + RTX 1000 mg	DMARD + BRM-IR	ACR20/50/70, DAS28
	REFLEX, Cohen et al.	520	24 weeks	MTX + RTX vs MTX + PLB	Anti-TNF-IR	ACR20, ACR50/ACR70, EULAR response, DAS28
	DANCER, Mease et al.	367	24 weeks	MTX + PLB vs MTX + RTX 500 mg vs MTX + RTX 1000 mg	DMARD + BRM-IR	SF-36, HAQ and FACIT-Fatigue scale, ACR20/50/70 and EULAR responses
	IMAGE, Tak et al.	755	52 weeks	MTX vs RTX 2 × 500 + MTX vs RTX 2 × 1000 + MTX	MTX-Naïve	GMSS, ACR50/70, MCR, DAS remission
	SUNRISE, Mease et al.	559	48 weeks Retreatment at week 24	RTX + MTX vs MTX + PLB	MTX + anti-TNF-IR	ACR20, ACR50/ACR70, DAS28, EULAR response
	MIRROR, Rubbert-Roth et al.	378	48 weeks, Retreatment at week 24	RTX 2x500 and 2x500 vs RTX 2x500 and 2x1000 (dose escalation) vs 2x1000 and 2x1000	MTX-IR	ACR20, ACR50/ACR70, DAS28, EULAR response, SF-36, HAQ and FACIT-Fatigue scale
	SERENE, Emery et al.	509	24 weeks	MTX + RTX 2x500 vs MTX + RTX 2x1000 vs MTX + PLB	MTX-IR	ACR20, ACR50/ACR70, DAS28, EULAR response, SF-36, HAQ and FACIT-Fatigue scale
	REFLEX, Keystone et al.	517	56 weeks	MTX + RTX vs MTX + PLB	Anti-TNF-IR	GMSS, ES, JSNS

TNF-inhibitor naïve patients and in patients who had failed to respond to these agents. Abatacept's efficacy tends to be seen later compared to anti-TNFs enduring beyond 6 months of treatment (Pham et al. 2012) (Table 5).

No specific laboratory or immunological tests are needed to monitor abatacept therapy. Standard practice laboratory tests to include inflammatory markers and liver function tests in cases of concomitant MTX therapy are indicated every 2 to 3 months. In the absence of comorbidities, cytopenias are uncommon (Pham et al. 2012).

Tyrosine Kinase Inhibitors

Despite the great advances in therapeutics, 30–40 % of RA patients do not achieve remission, highlighting the need for drugs with novel mechanism of action. Tyrosine kinases comprise of 90 members, among which Janus activation kinases (Jaks) and Spleen tyrosine kinase (Syk) have been implicated in RA pathogenesis, as evidenced by the successful preclinical studies with tofacitinib and fostamatinib. Jaks compose of Tyk2, Jak1, Jak2, and Jak3 which bind to type I and II receptors in different combinations. Upon ligand binding, Jaks phosphorylate cytokine receptors, initiating a signal transduction cascade which leads to gene transcription modulation (Kontzias et al. 2012).

Syk is a non-receptor cytoplasmic tyrosine kinase involved in signaling and the activation of Fc gamma receptors in many cells which are key players in RA pathogenesis. Of note Syk is

Rheumatoid Arthritis, Treatment, Table 5 Pivotal clinical trials of T-cell co-stimulation inhibitors in rheumatoid arthritis

T-cell co-stimulation inhibitors						
Agents	Clinical trial	# Pts	Study length	Intervention	Prior DMARD use	Significant outcome measures ($p \leq 0.05$)
Abatacept	ASSURE, Weinblatt et al.	1456	1 year	ABA vs PLB	DMARDs-IR	VAS, HAQ-DI
	AGREE, Westhovens et al.	509	1 year	ABA + MTX vs PLB + MTX	MTX-Naïve	DAS28, GMSS
	AIM, Kremer et al.	652	1 year	ABA vs PLB	MTX-IR	ACR20/50/70
	ATTAIN, Genovese et al.	738	6 months	DMARD + ABA vs DMARD + PLB	Anti-TNF-IR	ACR20/50/70, HAQ-DI
	ATTEST, Schiff et al.	748	1 year	ABA + MTX vs IFL + MTX vs MTX + PBL	MTX-IR	DAS28 (ESR based), HAQ-DI, ACR20/50/70
	ALLOW, Kaine et al.	167	36 weeks	ABA IV + MTX vs ABA SQ + MTX and ABA SQ + MTX vs PLB + MTX	MTX-IR	ELISA-detected immunogenicity rate and safety, DAS28, HAQ-DI

highly expressed by all hematopoietic lineage cells. Many kinase inhibitors with different specificities are in the pipeline for various indications in the spectrum of auto-immunity and cancer. As of now, tofacitinib, is approved by the FDA for treatment of moderate to severe rheumatoid arthritis in patients who have had an inadequate response to, or who are intolerant of, methotrexate. Fostamatinib's efficacy was not proven to be efficacious in three randomized placebo-controlled phase II trials and one phase III trial so this agent is no longer a candidate for RA treatment.

Tofacitinib

Tofacitinib (formerly designated CP-690,550) is the first selective Jak inhibitor to be tested in humans potently inhibiting Jak3 and Jak1 and to a lesser extent Jak2. It efficiently blocks common γc cytokines including IL-2, IL-4, IL-15 and IL-21, but it also inhibits signaling by IFN-γ, IL-6, and to a lesser extent IL-12 and IL-23, therefore abrogating Th1 and Th17 cellular effects. In vivo tofacitinib mitigates the effects of IL-6 and type I interferons on synovial fibroblasts, inhibiting chemokine expression (Kontzias et al. 2012).

The efficacy of tofacitinib in RA was established in phase II and III trials both as monotherapy and combined with methotrexate in patients who failed DMARDs. Tofacitinib was compared head to head with adalimumab and was found to be non-inferior. Importantly, there was evidence to suggest that structural damage was also prevented in the higher dose (10 mg twice daily) (Kontzias et al. 2012) (Table 6).

Safety signals from long extension studies include infections usually upper respiratory and less commonly opportunistic, mild increase in creatinine, hyperlipidemia, LFT abnormalities, neutropenia, and anemia.

Conclusion

The field of therapeutics in RA has leapt forward over the last 15 years. The "revolution of biologics" sustained great impact in the natural history of the most common autoimmune disease. This coincided with the better understanding of its pathogenesis which provided the rationale to target specific molecules found to play a critical role in the disease process. This effort would not

Rheumatoid Arthritis, Treatment, Table 6 Pivotal clinical trials of Janus kinase inhibitors in rheumatoid arthritis

Janus kinase (Jak) inhibitors						
Agents	Clinical trial	# Pts.	Study length	Intervention	Prior DMARD use	Significant outcome measures ($p \leq 0.05$)
Tofacitinib	Kremer et al	264	12 weeks	PLB vs. TOFA 5 mg vs. TOFA 15 mg vs. TOFA 30 mg	DMARDS + anti-TNF-IR	ACR20/50/70
	Kremer et al.	509	24 weeks		MTX-IR	ACR20/50/70, HAQ-DI
	Kanik et al.	384	6 months		DMARDS-IR	ACR20/50/70, DAS28
	Tanaka et al.	136	12 weeks		MTX-IR	ACR20/50/70

have been possible without the evolutionary tools of molecular biology. Multiple novel medications are in the pipeline targeting specific pathways with a great interest in those modulating signal transduction. Most of them are administered orally, potentially lowering the future health costs for the treatment of this chronic debilitating disease. Further comparative studies identifying predictors of response for subsets of patients are needed for a true tailored approach. It remains to be seen how RA's therapeutic algorithm will be changed in the future influenced by the currently established medications, the efficacy of novel drugs, and the future pharmacoeconomic environment.

Cross-References

- ▶ CTLA4-Ig
- ▶ Interleukin-6
- ▶ Rheumatoid Arthritis, Biologics in its Treatment
- ▶ Small Molecules Targeted For the Treatment of Rheumatoid Arthritis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Aaltonen KJ, Virkki LM, et al. Systematic review and meta-analysis of the efficacy and safety of existing TNF blocking agents in treatment of rheumatoid arthritis. PLoS One. 2012;7(1):e30275.

Benucci M, Manfredi M, et al. Predictive factors of response to rituximab therapy in rheumatoid arthritis: what do we know today? Autoimmun Rev. 2010;9(12):801–3.

Boyce EG, Halilovic J, et al. Golimumab: review of the efficacy and tolerability of a recently approved tumor necrosis factor-alpha inhibitor. Clin Ther. 2010;32(10):1681–703.

Buch MH, Emery P. New therapies in the management of rheumatoid arthritis. Curr Opin Rheumatol. 2011;23(3):245–51.

Canella AC, O'Dell J. Methotrexate, leflunomide, sulfasalazine, hydroxychloroquine, and combination therapies. Philadelphia: PA, Elsevier/Saunders; 2008.

Coury FF, Weinblatt ME. Clinical trials to establish methotrexate as a therapy for rheumatoid arthritis. Clin Exp Rheumatol. 2010;28(5 Suppl 61):S9–12.

Gaujoux-Viala C, Smolen JS, et al. Current evidence for the management of rheumatoid arthritis with synthetic disease-modifying antirheumatic drugs: a systematic literature review informing the EULAR recommendations for the management of rheumatoid arthritis. Ann Rheum Dis. 2010;69(6):1004–9.

Goekoop-Ruiterman YP, de Vries-Bouwstra JK, et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. Arthritis Rheum. 2005;52(11):3381–90.

Katz SJ, Russell AS. Re-evaluation of antimalarials in treating rheumatic diseases: re-appreciation and insights into new mechanisms of action. Curr Opin Rheumatol. 2011;23(3):278–81.

Kirwan JR. The effect of glucocorticoids on joint destruction in rheumatoid arthritis. The Arthritis and Rheumatism Council Low-Dose Glucocorticoid Study Group. N Engl J Med. 1995;333(3):142–6.

Kontzias A, Kotlyar A, et al. Jakinibs: a new class of kinase inhibitors in cancer and autoimmune disease. Curr Opin Pharmacol. 2012;12(4):464–70.

Maddison P, Kiely P, et al. Leflunomide in rheumatoid arthritis: recommendations through a process of consensus. Rheumatology (Oxford). 2005;44(3):280–6.

Markatseli TE, Papagoras C, et al. Prognostic factors for erosive rheumatoid arthritis. Clin Exp Rheumatol. 2010;28(1):114–23.
Mease PJ. Certolizumab pegol in the treatment of rheumatoid arthritis: a comprehensive review of its clinical efficacy and safety. Rheumatology (Oxford). 2011;50(2):261–70.
Mertens M, Singh JA. Anakinra for rheumatoid arthritis: a systematic review. J Rheumatol. 2009;36(6):1118–25.
Pham T, Bachelez H, et al. Abatacept therapy and safety management. Joint Bone Spine. 2012; 79(Suppl 1):3–84.
Singh JA, Beg S, et al. Tocilizumab for rheumatoid arthritis: a Cochrane systematic review. J Rheumatol. 2011;38(1):10–20.
Taylor PC. Pharmacology of TNF blockade in rheumatoid arthritis and other chronic inflammatory diseases. Curr Opin Pharmacol. 2010;10(3):308–15.
Wolfe F, Michaud K. Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. Arthritis Rheum. 2004;50(6):1740–51.

Rho/Rac GTPases

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Synonyms

Cdc42, cell division control protein 42 homolog, Cdc42Hs, G25K; Rac1, Ras-related C3 botulinum toxin substrate 1, p21-Rac1, TC25; Rac2, Ras-related C3 botulinum toxin substrate 2, EN-7, p21-Rac2, RacB; Rac3, Ras-related C3 botulinum toxin substrate 2, p21-Rac3, Rac1B; RhoA, *Aplysia* Ras homology A, *Aplysia* Ras homology A1, ArhA, ArhA1, Arh12, RhoH12; RhoB, *Aplysia* Ras homology B, ArhB, Arh6, p21-RhoB, RhoH6; RhoC, RhoA-related protein C, *Aplysia* Ras homology 9, Ras homolog 9, ArhC, Arch9, RhoH9, Silica-induced gene 61, Sig61; RhoD, *Aplysia* Ras homology D, Ras homolog D, Ras homolog gene family member D, ArhD, RhoHP1, RhoM; RhoG, *Aplysia* Ras homology G, Ras homolog G, ArhG; RhoH, Ras homolog gene family member H, ArhH, TTF; RhoJ, Ras homolog gene family member J, RhoI, RhoT, TC10-like Rho GTPase, TC21-like small GTPase, Tc101, TC10B, TC10L, TCL; RhoQ, Ras homolog gene family member Q, ArhQ, RhoQ, TC10A; RhoU, Ras homolog family member U, ArhU, Cdc42-like GTPase 1, Cdc42L1, GTP-binding protein-like 1, GTP-binding protein SB128, Ryu GTPase, SB128, Wnt1 responsive Cdc42 homolog 1, Wrch; RhoV, Ras homolog family member U, AhrV, Cdc42-like GTPase 2, Chp, GTP-binding protein-like 2, Wnt1 responsive Cdc42 homolog 1, Wrch1-related GTPase, Wrch2; Rif Alpha fetoprotein regulation 2, Afr2, ArhF, induced in fatty liver dystrophy 1, Ifld1, Ras homolog family member F, RhoF; Rnd1, ArhS, Rho family GTPase 1, Rho-related GTP-binding protein Rho6, Rho6, Rho6 GTP-binding protein; Rnd2, ArhN, Rho7, RhoN, RohN; Rnd3, Ras homolog family member E, AhrE, RhoE, Rho family GTPase 3, Rho8

Definition

This contribution provides a general overview about the biosynthesis cycle, regulatory mechanisms, and in vivo functions of Rho/Rac proteins, a large group of GTP-binding proteins belonging to the Ras superfamily, in both the immune system and immune-related diseases.

Historical Background

The first Rho/Rac family member (RhoA) was cloned serendipitously by R. Axel's group in 1985 while searching for human chorionic gonadotropin-like hormones in *Aplysia* using low-stringent cDNA library screenings. Rac1, Rac2, and Cdc42 were discovered in the 1989–1990 period using cDNA library screenings with degenerated oligonucleotides designed from peptides of the so-called G25K (Cdc42) GTPase. The rest of family members were discovered using a similar approach or, more recently, via the sequencing of the genomes of

Rho/Rac GTPases, Table 1 Rho/Rac GTPases

GTPase subfamily	Subfamily members
Rac	Rac1, Rac2, Rac3, RhoG
Rho	RhoA, RhoB, RhoC
Cdc42	Cdc42, RhoJ, RhoQ
RhoH	RhoH
RhoU/V	RhoU, RhoV
Rnd	Rnd1, Rnd2, Rnd3
RhoF	RhoD, RhoF

different species (Bustelo et al. 2007). Currently, this family is composed of 18 different genes that, by alternative splicing, can generate 22 proteins in humans (Bustelo et al. 2007). According to structural homology criteria, these proteins can be subclassified in seven different subfamilies (Table 1). Although some classifications include the three RhoBTB proteins (RhoBTB1, 2, and 3), they should not be considered bona fide members since they are quite different in terms of structure, function, and regulation.

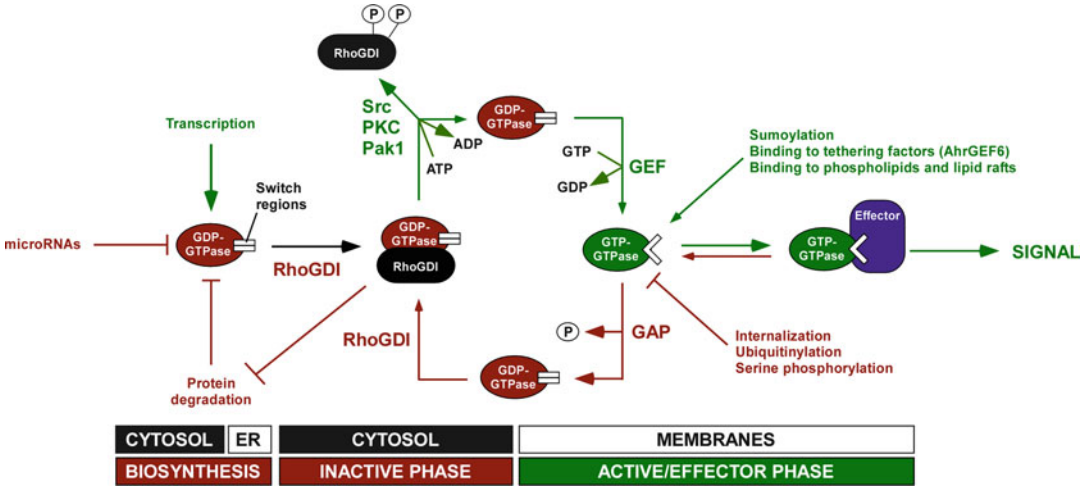
Like the majority of Ras superfamily proteins, the "conventional" Rho/Rac GTPases behave as molecular switches that fluctuate between inactive and active states depending on the signaling status of cells (Fig. 1). These states are modulated by the differential binding of GDP and GTP, two guanosine nucleotides that are essential for inducing the inactive and active state, respectively. When in the active state, these GTPases bind to a large collection of proximal elements (also referred to as "effector" molecules) that, in turn, promote the stimulation of downstream signaling cascades (Bustelo et al. 2007) (Fig. 1). However, a minority of "nonconventional" Rho/RacGTPases (i.e., Rnd subfamily members) are constitutively bound to GTP molecules and, therefore, are always present in a permanent active state. These proteins work as antagonists for conventional Rho/Rac GTPases (i.e., RhoA) (Chardin 2006). Conventional and nonconventional Rho/Rac GTPases control functions common to many cell types, including the regulation of the cytoskeletal structure, cell cycle transitions, cell survival, or vesicle trafficking (Bustelo et al. 2007; Chardin 2006). In addition, they are in charge of cell type-specific functions, including the differentiation, stimulation, migration, and chemotaxis of different immune cell types (Bokoch 2005; Bustelo 2002, 2007; Mulloy et al. 2010; Tybulewicz and Henderson 2009). In this entry, a brief discussion of the regulation and biological roles of this GTPase subfamily in specific cell lineages of the immune system will be presented. Such discussion includes information about (1) the biosynthetic cycle of Rho/Rac proteins, (2) the regulation of its GDP/GTP cycle, (3) the downstream effectors, (4) the biological routes controlled by them in immune cells that have been corroborated using animal models, (5) and the implication of these proteins in diseases related to the immune system. The implication of Rho/Rac proteins in other hematopoietic lineages and non-hematopoietic cells can be found elsewhere (Bokoch 2005; Bustelo et al. 2007; Mulloy et al. 2010).

Regulation

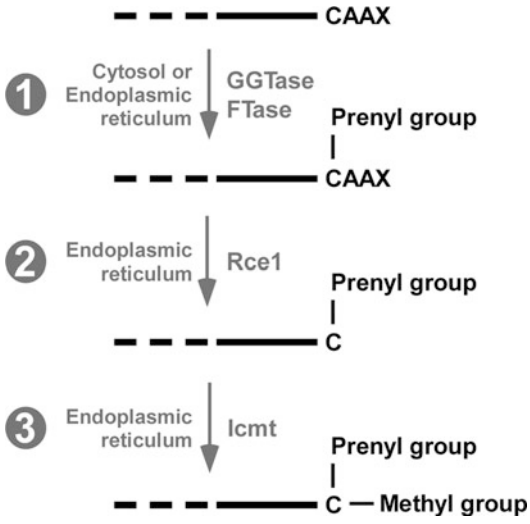
Rho/Rac GTPases are subjected to a complex array of regulatory mechanisms controlling their subcellular localization, activation level, and overall signaling output. Those include:

Posttranslational Modification of the C-Terminus. Most Rho/Rac proteins require the attachment to the plasma membrane and/or intracellular vesicles to carry out their biological functions. This step requires the posttranslational modification of Rho/Rac proteins in the four C-terminal amino acids that receive the collective name of "CAAX box" (where C is cysteine, A is any aliphatic amino acid, and X can be methionine, serine, alanine, or glutamine) (Bustelo et al. 2012, 2007). This modification takes place, depending on the GTPse involved, in three to four independent steps (Fig. 2):

(1) Incorporation of either a geranylgeranyl or a farnesyl group onto the cysteine residue of the CAAX box of the recently translated, cytosolic GTPase (Fig. 2, step 1). This process is catalyzed by either cytosolic type I geranylgeranyl transferases or endoplasmic-reticulum-localized farnesyl transferases. Geranylgeranylation is by far the



Rho/Rac GTPases, Fig. 1 *GTPase cycle*. Main regulatory mechanisms affecting the functional cycle of conventional Rho/Rac proteins. The GDP/GTP cycle as well as other regulatory steps mediated by the action of either effectors or other signaling elements is depicted in the figure. Activation and inactivation steps are indicated by *arrows* and *blunted lanes*, respectively. The subcellular localization and activation phases of the GTPase are indicated at the *bottom*. For the sake of simplicity, the regulatory cycle of a "conventional" Rho/Rac protein capable of undergoing GDP/GTP exchange has been only depicted. The regulatory steps for "nonconventional" Rho/Rac GTPases are similar, with the exception of the RhoGDI binding and GEF-/GAP-catalyzed steps. *ER* endoplasmic reticulum, *P* phosphate. Other abbreviations have been introduced in the main text



Rho/Rac GTPases, Fig. 2 *GTPase maturation*. C-terminal processing of Rho/Rac proteins. The palmitoylation step that some GTPases undergo upon C-terminal processing has not been depicted. See details in main text. *FTase* farnesyl transferase, *GGTase* geranylgeranyl transferase

most prevalent posttranslational event in Rho/Rac subfamily members, because farnesylation is only observed in RhoB, RhoJ, and RhoQ.

(2) Cleavage of the C-terminal AAX tripeptide at the endoplasmic reticulum by the action of Rce1, an isoprenyl, CAAX-specific endoprotease (Fig. 2, step 2).

(3) Methylation of the α-carboxyl group of the newly exposed C-terminal isoprenylcysteine residue of the GTPase at the endoplasmic reticulum, a process catalyzed by the carboxyl methyltransferase Icmt (Fig. 2, step 3).

(4) Some GTPases (i.e., Rac1, RhoB, RhoJ, RhoQ) are further modified by the addition of a palmitate group to cysteine residues situated immediately upstream of the CAAX motif. The enzyme responsible for this step is still uncharacterized in mammals.

Regulation by Rho GDP Dissociation Inhibitors (RhoGDIs). Once prenylated and processed at the endoplasmic reticulum, the mature

GDP-bound GTPases bind to RhoGDIs (Fig. 1). In fact, it is assumed that all the inactive pool of Rho/Rac GTPases is trapped in RhoGDI complexes. The main role of RhoGDIs is an inhibitory one, since they sequester the GTPases in the cytosol, restrict the release of GDP molecules from the bound GTPases, and extract the GTPases from membranes at the end of the stimulation cycle (Bustelo et al. 2012, 2007; DerMardirossian and Bokoch 2005) (Fig. 1). However, recent reports have revealed that RhoGDIs can also play positive roles in this route, favoring the translocation of these proteins during signal transduction and protecting the inactive GTPases from proteolytic degradation (Boulter et al. 2010; Bustelo et al. 2012; 2007; DerMardirossian and Bokoch 2005) (Fig. 1). Although the mechanism is not fully elucidated, it has been shown that the release of the GTPases from the RhoGDI complexes, a process required for the activation and membrane anchoring of the GTP-binding proteins, is mediated by the phosphorylation of RhoGDI by Src, protein kinase C, and Pak1 (Bustelo et al. 2012) (Fig. 1). There are three known RhoGDIs in vertebrates, all of them expressed in hematopoietic cells: RhoGDI1 (also known as RhoGDIα), RhoGDI2 (also known as D4/LyGDI/β), and RhoGDI3 (also referred to as RhoGDIγ) (DerMardirossian and Bokoch 2005).

Regulation of the GDP/GTP Cycle. The conventional members of the Rho/Rac family have to undergo GDP/GTP exchange in order to become activated during signal transduction. This process is catalyzed by enzymes known as Rho/Rac GDP/GTP exchange factors (GEFs) (Fig. 1). Depending on whether they contain Dbl-homology (DH) or Dock-homology catalytic domains, these GEFs are subclassified in Dbl and Dock subfamily proteins (Bos et al. 2007). Although mammals have nearly 70 proteins with this enzyme activity (Bos et al. 2007), the GEFs with specific roles in immune cells include, so far, only five Rac subfamily-specific GEF (Vav1, Vav2, Vav3, Tiam1, Dock2), a Rho subfamily-specific exchange factor (Lsc, also known as p115-RhoGEF and ArhGEF1), and a Rac1-/Cdc42-specific GEF (ArhGEF6, also known as α-Pix) (Tybulewicz and Henderson 2009). At the end of the activation cycle, the GTPases are inactivated by the action of the enzymes known as GTPase-activating proteins (GAPs). These proteins promote the hydrolysis of the GTPase-bound GTP molecules and, therefore, the transit back to the inactive, GDP-bound state of these GTPases (Bos et al. 2007) (Fig. 1). Similar to the Rho/Rac GEFs, there is a large number of GAPs in mammals (Bos et al. 2007), many of which are expressed in immune cells (Tybulewicz and Henderson 2009). However, few of them have been analyzed in the immune system using animal models (Tybulewicz and Henderson 2009).

Other Regulatory Steps. Recent work has been shown that the active GTPases can be regulated by additional mechanisms, including tethering to specific subcellular localizations, protein/protein interactions, ubiquitinylation, sumoylation, C-terminal phosphorylation, internalization/recycling using the vesicle trafficking machinery, transcriptional activation of their genes and microRNA-mediated degradation of GTPase-encoding transcripts (Fig. 1). Some of these mechanisms have been recently reviewed (Boulter et al. 2012; Bustelo et al. 2012, 2007; Chardin 2006).

Downstream Effectors

The activation of Rho/Rac proteins leads to their association with effector molecules responsible for the assembly of specific signaling cascades. To date, around 60 potential effectors have been identified for RhoA, Rac1, and Cdc42 that participate in different signaling pathways (Bustelo et al. 2007) (Table 2). Although this list is very extensive and highly heterogeneous from a functional point of view, the Rho/Rac effectors can be divided in eight main subclasses: (1) direct regulators of the F-actin cytoskeleton (Was, Baiap2, formins, rhotekins); (2) serine/threonine kinases controlling cytoskeletal

Rho/Rac GTPases, Table 2 Examples of effectors of the most important Rho/Rac GTPases

GTPase	Effector[a]
Rac1	ArhGEF7; Arfip2; Baiap2; CybA; Cyfip2; Fhod1; IQGAP1,2; Ncf1,2; Nck1; mTor1,2; Pak1; Pak2; Pak3; PIK3R1; Stat3; Wasf1,2
RhoA	Citron; Diaph1,2; Rtkn1; Rtkn2; Rhpn1; Rhpn2; PI-4-p5K; Pld1; Pkn1,2; Rock1,2
Cdc42	Baiap2; CopG2; Cdc42BPGA,B; Cdc42SE1,2; Cdc42EP1,3,5; Dia2,3; IQGAP1,2; Mig6; Pard6A,G; PIK3R1; Stat3, Tnk2; Was; Wasf1,2; WasL

[a]Names are given following the consensus Human Genome Organization nomenclature

dynamics and other downstream biological responses (Pak, Citron, Rock, and Pkn families); (3) tyrosine kinases (Tnk2, also known as Ack); (4) enzymes involved in lipid biosynthesis (▶ PI3K, PI-5-p5K); (5) proteins involved in the regulation of multisubunit complexes such as the neutrophil NADPH oxidase (Ncf1 and CybA, also known as $p47^{Phox}$ and $p22^{Phox}$, respectively); (6) proteins involved in cell polarity (i.e., Pard6A and B); (7) molecules that tether Rho/Rac proteins to specific subcellular localizations (i.e., ArhGEF7, Nck, CopG2); (8) and a heterogeneous group of proteins including metabolic enzymes (i.e., ▶ mTORC) and transcriptional factors (Stat3). The exact interactomes of all members of the Rho/Rac family are still unknown, so it is possible that additional effector molecules may exist. It is also important to note that highly related Rho/Rac GTPases share overlapping, but not identical, spectra of binding proteins. For example, Rac1, but not Rac2 or RhoG, can bind to members of the Pak serine/threonine kinase family.

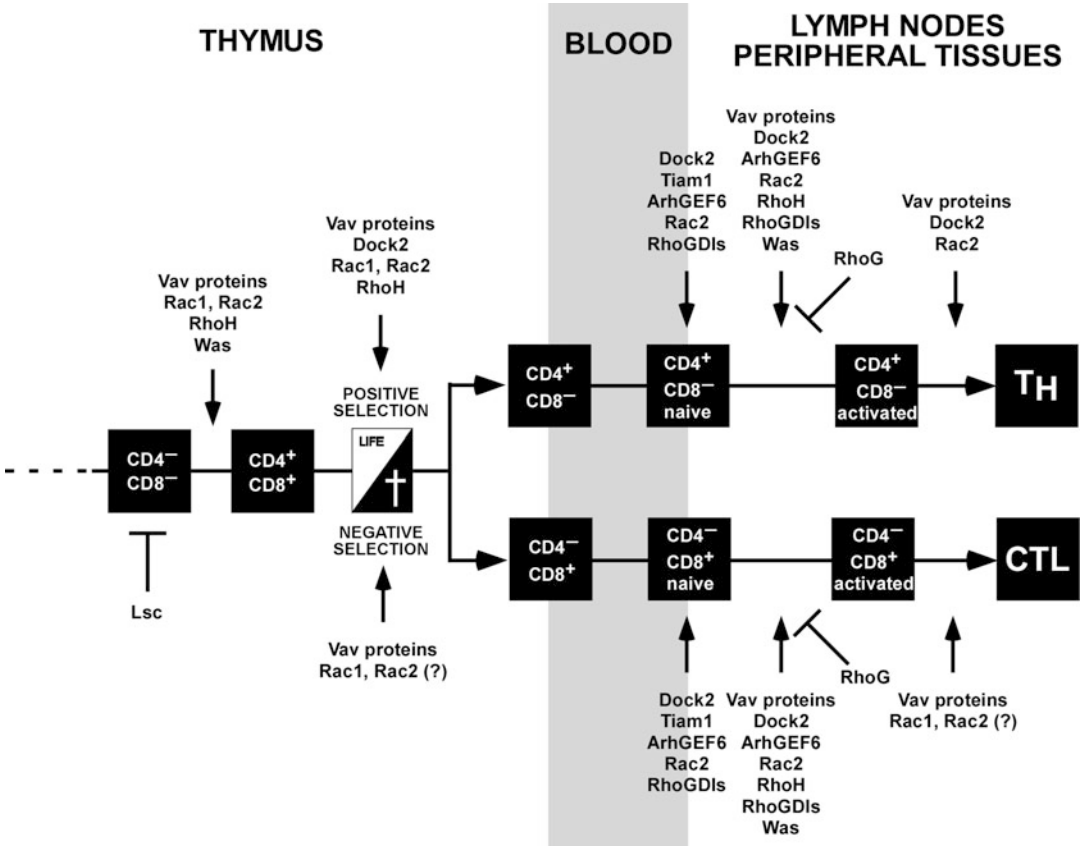
The interaction of Rho/Rac proteins with effectors is mediated by specific residues present in the switch I and switch II regions and, in some cases, further stabilized by interactions with other regions of the GTPase structure. This is consistent with the fact that the switch regions are the only parts of the GTPase molecule that undergo substantial conformational change upon the binding of guanosine nucleotides (Bustelo et al. 2007). However, in exceptional cases, the effectors can bind independently of the nucleotide-bound status of the GTPase. For example, mTorc1 and mTorc2, two signaling elements of the ▶ mTOR complex, can bind to indistinctly to the C-terminus of GDP- and GTP-bound Rac1 (Bustelo et al. 2012).

Upon binding, most of the effectors are activated (Bustelo et al. 2007). In the majority of cases, this is achieved by the induction of conformational changes that, in turn, favor the activation of the latent enzyme or biological activities of effectors (Bustelo et al. 2007). This is the case for the downstream enzymes belonging to the Pak, Rock, and Pkn families. In other cases, the activation step may relay exclusively in the tethering of the effector to specific subcellular localizations (Bustelo et al. 2007). Less frequently, Rho/Rac GTPases induce the release of inhibitors from a signaling complex (Bustelo et al. 2007). Thus, the activation of Wave1 by Rac1 is achieved by the indirect binding and release of Nap125 and Pir121 proteins from that complex. Finally, some interactions lead to the direct inhibition of the bound downstream effector (Bustelo et al. 2007; Chardin 2006). This is the case of Rnd3 that, by binding tightly to Rock, blocks the activation of this serine/threonine kinase by RhoA. The Cdc42-mediated inhibition of Borgs and the NADPH oxidase complex has also been described (Bustelo et al. 2007).

Biological Effects

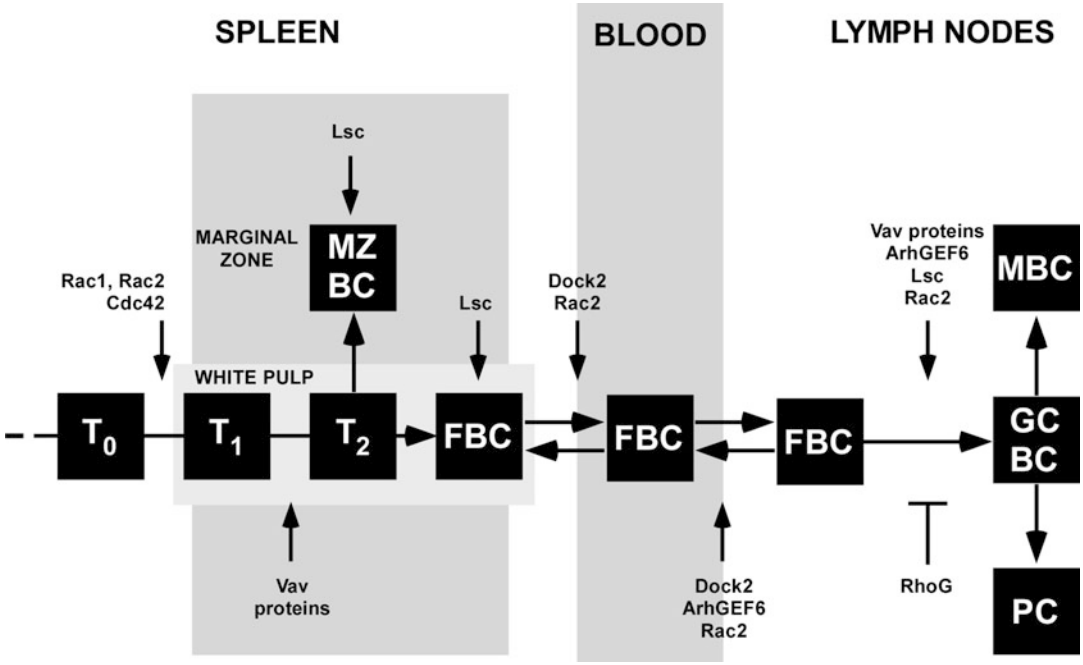
A wealth of information has been recently generated using knockout and knock-in animal models for specific signaling elements implicated in Rho-/Rac-dependent routes. The main biological processes controlled by those proteins in lymphocytes and myeloid cells are described below. The functions of Rho-/Rac-dependent routes in other hematopoietic cell types have been discussed in previous review articles (Bokoch 2005; Bustelo et al. 2007; Mulloy et al. 2010).

Rac Subfamily-Dependent Routes in T Lymphocytes. The most comprehensive characterization of the importance of Rac-dependent routes in this cell lineage has been done using animal



Rho/Rac GTPases, Fig. 3 *GTPases in T cells*. Schematic representation of T-cell development and the T-cell functions that are Rho/Rac GTPase dependent. Positive and inhibitory roles are indicated by *arrows* and *blunted lanes*, respectively. Putative functions are indicated by a *question mark*. *CTL* cytotoxic T cell, *TH* helper T cell, ✞ cell death

models deficient in Vav family members. These proteins work as tyrosine phosphorylation-dependent Rac subfamily-specific GEFs (Bustelo 2000; Bustelo 2008, 2010, 2011; Bustelo and Couceiro 2008; Turner and Billadeau 2002). These analyses revealed that Vav proteins play essential roles in both the development and effector functions of T cells. In the former case, those functions include favoring pre-T-cell receptor (TCR) signals for the proper transition of double negative ($CD4^-CD8^-$) into double positive ($CD4^+CD8^+$) thymocytes and in the positive/negative selection of $CD4^+CD8^+$ T cells (Fig. 3). Vav proteins also control effector functions of mature T cells downstream of the TCR that include, among others, the TCR capping and F-actin polymerization; the formation of the immune synapse, proliferation, and cytokine production; the induction of cytotoxic-, helper-, and T-cell-dependent B-cell responses; and the homing of stimulated T cells into peripheral tissues (Fig. 3). In addition to the expected effects on the F-actin cytoskeleton, Vav proteins control the activation of ▶ PI3K and phospholipase C-γ1 (PLC-γ1) and, therefore, the optimal activation of Ca^{2+} dependent routes, the PLC-γ1/RasGRP1/Ras/Erk pathway as well as Akt-, protein kinase C- and protein kinase D-dependent routes downstream of the TCR. These proteins are also required for the optimal stimulation of transcriptional factors such as ▶ NFAT, ▶ NFκB, and Foxo1. These functions involve the use of both



Rho/Rac GTPases, Fig. 4 *GTPases in B cells*. Schematic representation of B-cell development and the B-cell functions that are Rho/Rac GTPase dependent. Proteins with positive and negative roles are indicated by arrows and blunted lanes, respectively. T_0–T_2 immature B-cell stages, *FBC* follicular B cell, *MZBC* marginal zone B cell, *GCBC* germinal center B cell, *MBC* memory B cell, *PC* plasma cell

Rac GTPase-dependent and Rac GTPase-independent routes (Bustelo 2000, 2008, 2010; 2011; Bustelo and Couceiro 2008; Turner and Billadeau 2002; Tybulewicz and Henderson 2009). Other Rac subfamily exchange factors play more restricted roles in T cells. Thus, Dock2 has roles in the positive and negative selection of thymocytes and the TCR-triggered proliferation of mature T cells (Tybulewicz and Henderson 2009) (Fig. 3). It is possible that some of those functions are partially redundant to those engaged by Vav family proteins. Finally, the Rac subfamily-specific Tiam1 GEF favors the transendothelial migration of activated T lymphocytes (Tybulewicz and Henderson 2009) (Fig. 3).

The coordinated action of both Rac1 and Rac2 is required for T-cell development and positive selection. These roles are a copycat of those for the Vav family GEFs in the same developmental setting (Fig. 4). In mature T cells, the *Rac2* gene deficiency induces a similar although milder phenotype to that induced by the loss of the Vav GEF family. Finally, it has been shown that this GTPase is important for the differentiation of T-helper type 1 cells, probably by regulating the secretion of γ-interferon (Bustelo et al. 2007; Mulloy et al. 2010; Tybulewicz and Henderson 2009) (Fig. 3). Animals deficient in Rac1 and Rac2 have not been analyzed as yet in mature T cells, although it is predictable that the phenotype of those mice will result in the aggregation of defects previously detected in mice lacking expression of the aforementioned Rac GEFs. RhoG is dispensable for T-cell development, although its deficiency leads to slightly higher proliferation rates in stimulated T cells (Bustelo et al. 2007; Mulloy et al. 2010; Tybulewicz and Henderson 2009) (Fig. 3). RhoG has been also shown to be important for T-cell trogocytosis, a recently described biological process that promotes the internalization of large plasma membrane chunks of the antigen-presenting cell by the conjugated T cell (Martinez-Martin et al. 2011).

Rho- and Cdc42 Subfamily-Dependent Routes in T Lymphocytes. The function of Rho subfamily GTPases (RhoA, RhoB, RhoC) has not been analyzed comprehensively in T lymphocytes. The exception has been the characterization of mice deficient in Lsc, a Rho subfamily-specific GEF-activated downstream of G-coupled receptors. In contrast to Rac subfamily GEFs, the lack of Lsc leads to an increase in the total numbers of mature T cells, a phenotype that has been linked to deficient apoptosis of immature T cells downstream of the thromboxane receptor (Tybulewicz and Henderson 2009) (Fig. 3). The role of Cdc42 subfamily proteins in T cells has not been explored either. However, the Rac1-/Cdc42-specific ArhGEF6 has been shown to be important for the proper activation of the Pak family of serine/threonine kinases downstream of the T-cell receptor and for the formation of cell conjugates with dendritic cells (Tybulewicz and Henderson 2009). The analysis of mice lacking Was, a Cdc42 effector, has demonstrated that this protein is critical for the normal production of immature and mature T cells (Fig. 3). The reduced production of thymocytes is due to impaired progression from the $CD44^{-}CD25^{+}$ to the $CD44^{-}CD25^{-}$ differentiation stage (Bustelo et al. 2007). *Was*$^{-/-}$ thymocytes and mature T cells show impaired T-cell receptor capping and endocytosis, defective Ca^{2+} fluxes, and reduced F-actin levels. As a consequence, they proliferate poorly upon engagement of the T-cell receptor (Bustelo et al. 2007) (Fig. 3).

Role of other Rho/Rac GTPase Subfamilies in T Lymphocytes. The analysis of *Rhoh*$^{-/-}$ mice has shown that this GTPase has also roles in T-cell development. This role seems to be connected to an adaptor function that favors the translocation of Zap70, a tyrosine kinase that plays essential roles downstream of the T-cell receptor (Tybulewicz and Henderson 2009) (Fig. 3).

Rac Subfamily-Dependent Routes in B Lymphocytes. Vav proteins play roles in B-cell differentiation, in pre-B-cell receptor-triggered proliferation, and in many antigen receptor-induced mature B-cell responses (i.e., proliferation, immune synapse formation, adhesion, spreading, peripheral survival) (Fig. 4). Vav proteins also work downstream of the receptors for polysaccharide (LPS) and the CCL21, CXCL13, and CXCL12 ▶ chemokines (Bustelo 2000, 2008, 2010, 2011; Bustelo and Couceiro 2008; Turner and Billadeau 2002; Tybulewicz and Henderson 2009) (Fig. 4). Dock2 is important for chemokine-induced B-cell migration, leading to defective infiltration of B cells in lymph nodes and the splenic white pulp. It also favors the adhesion and transendothelial migration of B cells through lymph node capillaries as well as the migration out of lymph nodes in response to sphingosine-1-phosphate stimulation (Tybulewicz and Henderson 2009) (Fig. 4).

Mice lacking Rac1 and Rac2 proteins display a developmental block of B-cell development at very immature stages, a phenotype connected to the improper activation of Akt-dependent survival signals and the poor expression the antiapoptotic BAFF receptor and Bcl-x_L (Fig. 4). In addition, immature B cells lacking these two GTPases cannot migrate properly, leading to a defective infiltration of the splenic white pulp and further impairments of the survival and maturation of these cells (Bustelo et al. 2007; Tybulewicz and Henderson 2009) (Fig. 4). *Rhog*$^{-/-}$ B cells can develop normally. However, as in the case of T lymphocytes (see above), RhoG-deficient mature B cells hyperproliferate upon B-cell receptor stimulation when compared to control cells (Bustelo et al. 2007; Tybulewicz and Henderson 2009) (Fig. 4).

Rho- and Cdc42 Subfamily-Dependent Routes in B Lymphocytes. Little information is available regarding the role of Rho subfamily GTPases in this cell lineage. The only exception has been the analysis of Lsc-deficient mice, which has revealed an implication of this RhoA GEF in the generation of normal B-cell numbers, the engagement of T-cell-dependent B-cell responses, and the LPS-induced migration of B cells to follicles (Tybulewicz and Henderson 2009) (Fig. 4). In the case of Cdc42, the recent analysis of inducible Cdc42 knock-in mice has revealed that this GTPase is important for the development of B1a and conventional B cells, probably by regulating pro-survival signals downstream of the B-cell

receptor (Bustelo et al. 2007; Mulloy et al. 2010; Tybulewicz and Henderson 2009) (Fig. 4). The use of knockout mice for ArhGEF6 indicates that this protein is important for the migration of B cells to lymph nodes and B-cell receptor-triggered proliferation (Tybulewicz and Henderson 2009) (Fig. 4). Thus, the activation of Cdc42 in immature B cells is probably regulated by other Rho/Rac GEFs.

Rac Subfamily-Dependent Routes in Myeloid Cells. Vav proteins are important for integrin-dependent adhesion and spreading, phagocytosis, and the oxidative burst of neutrophils. These functions contribute to the optimal assembly of IgG-/FcγR-mediated hemorrhage and edema responses in lung and skin, the interstitial transit of those cells toward sites of bacterial infection, and the effective assembly of responses against nosocomial organisms (Bustelo 2008, 2010, 2011, Bustelo and Couceiro 2008; Mulloy et al. 2010). Rac1 and Rac2 play nonoverlapping roles in these cells. Thus, Rac2 is critical for the motility, chemotaxis, phagocytosis, and the activation of the NADPH oxidase complex. Rac1 seems to be only important for chemokine-dependent responses. Rac1 and RhoG also participate, although in a less significant manner than Rac2, in the activation of the NADPH oxidase complex (Bustelo et al. 2007; Mulloy et al. 2010; Tybulewicz and Henderson 2009).

In the case of macrophages, Vav1 and Vav3 are important for ROS production and, in addition, for complement-mediated phagocytosis. The latter role seems to be connected to the proper tethering of Arp2/3 complex and F-actin to the phagocytic cup (Bustelo 2008, 2010, 2011, Bustelo and Couceiro 2008; Mulloy et al. 2010). Rac2 is important for both migration and superoxide production and phagocytosis to some (i.e., FcγR stimulation, IgG-sensitized sheep red blood cells) stimuli. By contrast, Rac1 seems to be important for regulating macrophage cell morphology and lamellipodia formation (Bustelo et al. 2007; Mulloy et al. 2010; Tybulewicz and Henderson 2009).

Other Rho/Rac Subfamily-Dependent Routes in Myeloid Cells. Cdc42 is involved in neutrophil polarity, as inferred from recent data using both Cdc42- and Cdc42-GAP-deficient cells (Mulloy et al. 2010). In addition, Wasp-deficient neutrophils have reduced phagocytic activity (Bustelo et al. 2007). The role of other Rho/Rac GTPases in this cell lineage is still unknown.

Rho-/Rac-Dependent Routes in Human Immune Diseases

An inactivating mutation in Rac2 (Asp57→Asn) with a dominant negative effect has been associated with a severe neutrophilic immunodeficiency in humans, a disease phenotypically very similar to the phenotype of *Rac2*$^{-/-}$ mice in this cell lineage (Mulloy et al. 2010). Different mutations in the *Was* gene, a locus encoding the Cdc42 Was effector that plays critical roles in cytoskeletal architecture, are the molecular cause for the recessive X-linked Wiskott-Aldrich immunodeficiency syndrome (WAS) that affects lymphocyte and myeloid cell function. T and B cells from WAS patients have both altered and reduced numbers of microvilli in their surface and, in addition, display defective downstream signaling from antigen B- and T-cell receptors (Bustelo et al. 2007). In cancer, the *RHOH* locus is frequently involved in chromosomal translocations in cases of human multiple myeloma, non-Hodgkin lymphoma, and B-cell chronic lymphocytic leukemia (Mulloy et al. 2010). Rac subfamily proteins are important for the tumorigenic status of chronic myeloid and MML-AF9-derived leukemia, as assessed by the use of Rac family animal models and Rac-specific inhibitors (Mizukawa et al. 2011; Mulloy et al. 2010). Finally, it has been shown that the immunodeficiency virus (HIV) stimulates the Rac1 route to favor its infectivity cycle. To this end, HIV encodes a protein (Nef) that, upon expression in the infected T cells, promotes the activation of the Rac1/Pak1 route through the direct binding to Pak1 and Rac1 GEFs (Vav1 and Dock2) (Greenway et al. 2003).

Conclusion

The studies conducted so far have shown that Rho/Rac GTPases play critical roles in the

development, selection, differentiation, and function of many immune-related cells such as lymphocytes and myeloid cells. Information gathered during these years has also indicated that Rho-/Rac-dependent signaling elements could be potential therapeutic targets to deal with immune dysfunctions and immune-related neoplasias. However, this is probably just the tip of the iceberg, since only a very limited subset of Rho/Rac signaling elements have been characterized so far at the functional and genetic level in immune cells. Further work in this area will yield additional information about the role of this complex protein family in the development and functionality of the immune system.

Cross-References

- ▶ Cell Adhesion Molecules
- ▶ Chemokines
- ▶ Mammalian Target of Rapamycin (mTOR)
- ▶ NF-κB
- ▶ Nuclear Factor of Activated T Cells (NFAT)
- ▶ PI3K

References

Bokoch GM. Regulation of innate immunity by Rho GTPases. Trends Cell Biol. 2005;15:163–71.

Bos JL, Rehmann H, Wittinghofer A. GEFs and GAPs: critical elements in the control of small G proteins. Cell. 2007;129:865–77.

Boulter E, Garcia-Mata R, Guilluy C, Dubash A, Rossi G, Brennwald PJ, Burridge K. Regulation of Rho GTPase crosstalk, degradation and activity by RhoGDI1. Nat Cell Biol. 2010;12:477–83.

Boulter E, Estrach S, Garcia-Mata R, Feral CC. Off the beaten paths: alternative and crosstalk regulation of Rho GTPases. FASEB J. 2012;26:469–79.

Bustelo XR. Regulatory and signaling properties of the Vav family. Mol Cell Biol. 2000;20:1461–77.

Bustelo XR. Understanding Rho/Rac biology in T-cells using animal models. Bioessays. 2002;24:602–12.

Bustelo XR. Vav family. In: Choy S, editor. Encyclopedia of signaling molecules. Vol. 2011, Springer; p in press; 2011.

Bustelo XR. Vav1. UCSD Nature Mol Pages 2010: doi:10.1038/mp.a002360.01.

Bustelo XR. Vav2. UCSD Nature Mol Pages 2008: (DOI: 10.1038/mp.a002361.01).

Bustelo XR and Couceiro JR. Vav3. UCSD Nature Mol Pages 2008: (DOI: 10.1038/mp.a002362.01).

Bustelo XR, Sauzeau V, Berenjeno IM. GTP-binding proteins of the Rho/Rac family: regulation, effectors and functions in vivo. Bioessays. 2007;29:356–70.

Bustelo XR, Ojeda V, Barreira M, Sauzeau V, Castro-Castro A. Rac-ing to the plasma membrane. The long and complex work commute of Rac1 during cell signaling. Small Gtpases. 2012;3:1–7.

Chardin P. Function and regulation of Rnd proteins. Nature Rev. 2006;7:54–62.

DerMardirossian C, Bokoch GM. GDIs: central regulatory molecules in Rho GTPase activation. Trends Cell Biol. 2005;15:356–63.

Greenway AL, Holloway G, McPhee DA, Ellis P, Cornall A, Lidman M. HIV-1 Nef control of cell signalling molecules: multiple strategies to promote virus replication. J Biosci. 2003;28:323–35.

Martinez-Martin N, Fernandez-Arenas E, Cemerski S, Delgado P, Turner M, Heuser J, Irvine DJ, Huang B, Bustelo XR, Shaw A, Alarcon B. T cell receptor internalization from the immunological synapse is mediated by TC21 and RhoG GTPase-dependent phagocytosis. Immunity. 2011;35:208–22.

Mizukawa B, Wei J, Shrestha M, Wunderlich M, Chou FS, Griesinger A, Harris CE, Kumar AR, Zheng Y, Williams DA, Mulloy JC. Inhibition of Rac GTPase signaling and downstream prosurvival Bcl-2 proteins as combination targeted therapy in MLL-AF9 leukemia. Blood. 2011;118:5235–45.

Mulloy JC, Cancelas JA, Filippi MD, Kalfa TA, Guo F, Zheng Y. Rho GTPases in hematopoiesis and hemopathies. Blood. 2010;115:936–47.

Turner M, Billadeau DD. VAV proteins as signal integrators for multi-subunit immune-recognition receptors. Nat Rev Immunol. 2002;2:476–86.

Tybulewicz VL, Henderson RB. Rho family GTPases and their regulators in lymphocytes. Nat Rev Immunol. 2009;9:630–44.

Role of B Cells in Suppressing/Modulating Immune Function

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Synonyms

Bregs

Definition Based on Function

Regulatory B cells are B lymphocytes that are capable of impairing or suppressing one or more components of an immune reaction (Mauri and Bosma 2011). B cell production of antibody subclasses with high affinity for inhibitory Fc receptors may be considered one suppressive function of B cells. However, the term regulatory B cell ("Breg") is most frequently reserved to describe antibody-independent mechanisms of suppression. Such suppressive activity can occur either directly by producing cytotoxic and suppressive soluble factors or indirectly by enabling other immune cells with suppressive properties.

One indirect mechanism is antigen presentation by normal naïve B cells to promote the development of regulatory T cells ("Tregs") (Redfield et al. 2011). Depleting Tregs in mice results in poor growth of transferred tumors, likely through enhanced responses of other T cells with inflammatory, antitumor properties (Tadmor et al. 2011). In this model, Treg-phenotype ($CD25^+Foxp3^+$) cell proliferative capacity and suppressive activity are suboptimal if the tumor recipients cannot produce B cells (Tadmor et al. 2011). Furthermore, B cells promote the accumulation of Treg-phenotype cells in the spleen, tumor-draining lymph node, and tumor bed, associated with reduced cytotoxic spleen cell activity, IFN-γ secretion, and enhanced growth of the injected tumor (Tadmor et al. 2011). This evidence collectively suggests that Bregs promote the differentiation and/or expansion of Tregs, which in turn suppress cytotoxic and inflammatory reactions that otherwise disable tumor growth. Thus, targeting the Breg-Treg axis may be a way to enhance immunotherapies against malignancy.

Direct suppressive mechanisms include the ability of B cell chronic lymphocytic leukemia cells to express the cytotoxic molecule granzyme B and kill other B-CLL cells in vitro (Jahrsdorfer et al. 2006). Additionally, normal (non-transformed) activated human B cells can reduce T cell division (Bouaziz et al. 2010). B cells can also secrete soluble factors that reduce expression of inflammatory cytokines by T cells. For example, supernatants of IFN-β-treated human B cells reduce *IL17* mRNA levels and IL17 protein secretion from activated T cells, an effect that can be reversed by neutralizing IL27 (Ramgolam et al. 2011). Therefore, IFN-β causes B cells to secrete something that inhibits T cell IL17 production in an IL27-dependent manner. Similarly, B cell suppression of the inflammatory cytokines TNF-α and IFN-γ in T cells can be reversed by neutralizing interleukin 10 (IL10) in culture (Blair et al. 2010; Bouaziz et al. 2010; Iwata et al. 2011). After *Salmonella* bacterial infection in vivo, mouse B cell IL10 can limit accumulation of TNF-α^+ neutrophils, IFN-γ^+ NK cells, IFN-γ^+ CD4 T cells, TNF-α^+ CD4 T cells, and IFN-γ^+ CD8 T cells in spleen as well as inflammatory foci in liver, correlating with inhibition of bacterial clearance and inhibition of mouse survival (Maseda et al. 2012). Collectively, these observations demonstrate that B cells have suppressive activity in vivo and suggest a mechanism in which such cells dampen inflammatory responses mediated by T cells as well as innate immune cells that otherwise protect from lethal pathology induced by microbial pathogens.

B Cell IL10 Induction

IL10 production is the most well-studied suppressive mechanism in human B cells, and its potential for resolving immune reactions is corroborated by evidence from mouse studies. Like many other cytokines, IL10 expression by B cells is undetectable directly ex vivo without additional stimulation. However, when human spleen, tonsil, adult blood, or cord blood mononuclear cells are cultured for ~5 h with toll-like receptor (TLR) ligands in the presence of PMA/ionomycin, ~0.5–3 % of the B cells become $IL10^+$ (Bouaziz et al. 2010; Iwata et al. 2011). Similar results are observed for mouse B cells (Yanaba et al. 2009). This readout is inferred to represent an immediate capacity to produce IL10 in vivo, such that Iwata et al. referred to the cells as the "B10" subset and distinguished "B10" from "B10 progenitor" to refer to cells that

produce the cytokine only after stimulation on the order of days (Iwata et al. 2011). In this case, a boost with stimuli such as PMA/ionomycin in the final few hours is essential (Iwata et al. 2011) and is included in the studies described hereafter. Caution should be noted, however, because the term "B10" is inconsistently used to indicate any $IL10^+$ B cell detected after such longer stimulations.

Human B cell IL10 production induced by TLR ligands like LPS or CpG can be further enhanced by signaling through CD40 (Iwata et al. 2011; Yanaba et al. 2009), and CD40 engagement by itself also stimulates a range of IL10 secretion from purified human B cells (Duddy et al. 2007). Thus, T cell-independent (TLR ligation) and T cell-derived (CD40 ligation) signals alone or in combination can promote B cell production of IL10.

Two human studies show that engaging the B cell receptor (BCR) before or during treatment with CD40L ± CpG can reduce IL10 production, but not IL10 production induced by CpG alone (Duddy et al. 2007; Iwata et al. 2011). However, another study showed that BCR engagement could synergize with CpG to increase IL10 secretion (Bouaziz et al. 2010). It remains to be determined whether technical differences such as the specific anti-BCR reagent or the readout of accumulated secreted cytokine in the latter study *versus* intracellular staining in the former accounts for this apparent discrepancy. Nonetheless, these observations suggest that IL10 production by B cells may be regulated by the co-stimulation available when the cell encounters its cognate antigen and by the circumstances of B cell receptor engagement.

Besides the stimuli described above, IL10 as well as IL27 and IL12 secretion by human B cells can be induced by IFN-β, a treatment that also decreases B cell IL1β and IL23 secretion (Ramgolam et al. 2011). IL10 production by individual human B cells in culture is typically mutually exclusive with that of other inflammatory cytokines including IFN-γ, IL6 (Amel Kashipaz et al. 2003), and lymphotoxin (Duddy et al. 2007). $IL10^+$ B cells from human CD19 transgenic mice do not have a substantial enrichment for any particular cytokine message besides IL10 (Yanaba et al. 2009). These observations collectively suggest that IL10 production by B cells occurs in a relatively polarized fashion in which co-expression of inflammatory cytokines (besides IL12) are disfavored. For this reason, "B10" B cells are commonly thought of as a distinct functional subset or collection of subsets.

Neonatal mice have larger proportions of IL10-competent B cells compared with adults (Yanaba et al. 2009). The importance of this difference for immune system development remains to be determined. Interestingly, IL10-competent B cells from unmanipulated adult mice have diverse immunoglobulin gene usage, ≥80 % of them lack immunoglobulin somatic hypermutations, and ~70 % are not antibody isotype switched (Maseda et al. 2012). Thus, at least in mice at steady state, many IL10-expressing cells may be induced without specific antigen selection or participation in organized germinal centers. Consistent with this conclusion, gnotobiotic mice have similar frequencies and numbers of IL10-competent B cells compared with mice raised in conventional facilities (Yanaba et al. 2009). Nonetheless, injecting mice with stimuli like LPS can increase the frequency of $IL10^+$ splenic B cells by 3 days (Maseda et al. 2012). Although studies of completely germ-free mice are needed to establish the true contribution of environmental microbial molecules in determining steady-state B10 numbers, the evidence thus far indicates that de novo microbial stimuli can trigger inducible IL10-competent B cells.

Mouse genetic studies have also helped define in vivo B cell IL10 regulation by host molecules. Activity of the mouse IL10 gene regulatory elements are unaffected by IL10 expression itself (Maseda et al. 2012). Thus, any feedback autocrine regulation of this cytokine does not appear to occur at the transcriptional level in mice. Optimal steady-state numbers of IL10-competent B cells do not require CD21, MHC class II, or CD40 (Yanaba et al. 2009), indicating that CD21 complement receptor signals and T cell help are dispensable. In fact, T cells as well as the B cell surface protein CD22 can limit IL10-competent

B cells detected in mouse lymphoid organs (Yanaba et al. 2009). It remains to be determined whether the apparent discrepancy between T cell signals (CD40 engagement) being able to induce IL10 in vitro and the limiting effect of T cells in vivo could be due to different T cell subsets providing differential regulation to distinct B cell subsets (discussed below) in various circumstances.

Optimal steady-state numbers of IL10-competent mouse B cells require an intact B cell receptor repertoire, the B cell receptor co-stimulatory molecule CD19, as well as expression of the TLR signaling molecule myd88 (Yanaba et al. 2009). Myd88 has multifaceted roles in antimicrobial responses. On one hand, mice globally deficient in myd88 demonstrate that this molecule is needed for protection from *Salmonella* infection (correlating with splenic neutrophil and IFN-γ^+ NK cell numbers) (Maseda et al. 2012). Myd88 in B cells is important for optimal kinetics of the antimicrobial B cell response (GC-phenotype cells, antibody titers, and long-lived plasma cells) (Maseda et al. 2012). However, B cell myd88 also suppresses inflammatory IFN-γ^+ CD4, TNF-α^+ CD4, and IFN-γ^+ CD8 T cell, TNF-α^+ neutrophils, and IFN-γ^+ NK cell accumulation in spleen, limits inflammatory foci in liver, and suppresses bacterial clearance and mouse survival (Maseda et al. 2012). Thus, TLR signaling in B cells appears to have a dual function of promoting antimicrobial responses as well as resolving the ensuing inflammation induced by the pathogen.

IL10-Competent B Cell Subsets

The observations described above demonstrate that suppressive activity and IL10 production by B cells are complex in their regulation. Optimally utilizing this information for therapeutic advantage could be enhanced by understanding specifically which phenotypic B cell subsets are responsible for these functions in different circumstances. Most commonly defined human B cell subsets (Anolik et al. 2009) are capable of producing IL10 (Blair et al. 2010; Bouaziz et al. 2010; Iwata et al. 2011). Nonetheless, considerable investment has been made in determining the relative capacity of these subsets for IL10 production in different situations.

Transitional B cells are defined as recent emigrants from bone marrow into peripheral circulation and secondary lymphoid organs where they complete maturation (Anolik et al. 2009). These B cells are enriched in neonatal humans and are often the first to repopulate the circulation of adults that have been therapeutically depleted of their B cells. Compared with more mature human B cell subsets, these cells co-express higher levels of CD24 and CD38 (Blair et al. 2010). In culture, $CD24^{high}CD38^{high}$ human B cells can inhibit T cell production of TNF-α by a mechanism involving IL10 secretion and CD80/86 interactions with T cells (Blair et al. 2010). This B cell fraction can also inhibit T cell production of IFN-γ by a CD80/86-mediated mechanism that appears to be less dependent on IL10 (Blair et al. 2010). Some groups have found that 5-h stimulation of human peripheral blood mononuclear cells with CpG yields the highest proportion of $IL10^+$ cells from the $CD24^{high}CD38^{high}$ fraction compared with other B cells (Bouaziz et al. 2010). Similar results were observed when the cells are sorted before 3-day stimulation through CD40 (Blair et al. 2010). Whether enrichment of transitional-phenotype B cells accounts for the high frequencies of IL10-competent B cells in neonatal mice and whether this is also the case for humans is unknown. Others have demonstrated that IL10 secretion after anti-CD40 (± anti-BCR) is enriched in the $CD27^{neg}$ B cell fraction (Duddy et al. 2007), known to contain both transitional and mature-naïve B cells. In fact, enhanced IL10 production from reconstituted B cells in neuromyelitis optica patients previously treated with B cell depletion therapy correlates with excess $CD27^{neg}$ B cells (Duddy et al. 2007). Collectively, these data show that B cells characteristic of early differentiation stages can be a source of IL10. It is not entirely clear if the common phenotype represents early B cells that are suppressive or if some suppressive B cells happen to

share a cell surface phenotype with early B cells. Nonetheless, the observation that early reconstitution of transitional-phenotype B cells correlates with long-term clinical remission in SLE patients previously treated with B cell depletion therapy (Anolik et al. 2007) suggests that the immunosuppressive potential of such cells could be harnessed through specialized therapies.

Human B cells positive for the activation/memory marker CD27 are also capable of producing IL10 (Bouaziz et al. 2010; Iwata et al. 2011) correlating with the ability to suppress T cell and monocyte TNF-α production (Blair et al. 2010). Reports appear to differ on whether such cells compared with the earlier phenotypic subsets are the most efficient IL10 producers. Whether the apparent discrepancies result from analytical variation or truly represent biological differences among subject groups and immunological circumstances remains to be resolved.

Evidence from mouse studies also suggests efficient IL10 production from activated B cells, as indicated by higher proportions of $IL10^+$ B cells expressing the marker CD43 (Maseda et al. 2012; Yanaba et al. 2009). A fraction of $CD43^+IL10^+$ mouse B cells are $GL7^+$ (Maseda et al. 2012), suggesting that germinal center B cells can express IL10. This may account for or at least contribute to the ~20 % of $IL10^+$ B cells with immunoglobulin somatic hypermutation and ~30 % that are isotype-switched (Maseda et al. 2012). The remaining $IL10^+$ cells may derive from locations and/or B cell fractions that are not in typical follicles associated with germinal center formation. Consistent with this suggestion, mouse $IL10^+$ cells have more CD5, CD1d, and CD19, but less CD23 compared with IL10-negative B cells (Maseda et al. 2012; Yanaba et al. 2009). This phenotype is characteristic of cells in the splenic marginal zone and also B1 B cells that are considered "innate-like" based on their rapid responsiveness to stimuli and use of germline B cell receptor sequences (Martin and Kearney 2002). $IL10^+$ B cells from adult T cell-deficient nude mice are all $CD1d^{high}CD5^+$, whereas in T cell-competent mice, it is only a 70 % majority (Yanaba et al. 2009). Thus, IL10 induced in innate-like phenotype B cells is T cell independent, whereas IL10 from other B cell subsets is likely influenced by signals from T cells.

Intriguingly, evidence from mice suggests that some IL10 production is from B cells that have differentiated to secrete antibody. At steady state, a fraction of $CD43^+IL10^+$ mouse B cells are $B220^{low}$ (Maseda et al. 2012), a characteristic of antibody-secreting cells. Similarly, 1 day after injecting IL10 mRNA reporter mice with LPS, IL10 reporter gene signal can be found in 16 % of cells with the plasma cell phenotype $B220^{low}CD138^+$ (Maseda et al. 2012). At the transcript level, purified mouse $CD1d^{high}CD5^+$ B cells are enriched for *IL10* as well as *blimp1* and *IRF4* mRNA when compared with $CD1d^{neg}CD5^{neg}$ B cells (Maseda et al. 2012). Another reporter model revealed that B cells capable of expressing IL10 are enriched for *blimp1* and *XBP1* mRNA, but *Pax5* and *Bcl6* levels are lower (Maseda et al. 2012). These gene expression profiles are characteristic of antibody-secreting plasma cells and plasmablasts. Functionally, mouse B cells expressing an IL10 mRNA reporter gene are more likely to secrete antibody in vitro and reconstitute antibody in lymphocyte-deficient mice in vivo compared with reporter gene-negative B cells (Maseda et al. 2012). IL10-expressing antibody-secreting cells can be induced by either immunization with haptenated protein or injection with attenuated *Salmonella* bacteria (Maseda et al. 2012). The true importance of this B cell functional multitasking remains to be fully understood. Differentiation of IgM secretion from $CD1d^{high}CD5^{neg}$ B cells after LPS treatment in vivo does not require IL10 expression (Maseda et al. 2012). However, IgM derived from IL10 reporter-positive B cells transferred into lymphocyte-deficient mice is polyreactive against TNP, dsDNA, ssDNA, and histones (Maseda et al. 2012). This was not the case for IgG derived from the same pool of IL10-competent cells. It remains to be determined at what level the regulation of this self-reactivity *versus* effector isotype is regulated, which cells are responding to IL10, and the overall contribution of this system to peripheral tolerance.

Bregs in Disease

B cell suppressive activity may be advantageous in dampening damaging inflammation once a pathogen is successfully cleared. However, this activity may also contribute to the pathogen's ultimate success (Maseda et al. 2012; Neves et al. 2010). Thus, vaccine design could benefit from trying to surpass such suppressive activity while enhancing protective reactions. Further elaboration of the in vitro and in vivo regulation of different B cell subsets with suppressive potential will be needed to catalyze this optimization.

Reciprocally, this knowledge may also be used to benefit individuals that suffer from self-targeted immune reactions. For example, helminth (parasitic worm) infection increases the number of $IL10^+$ B cells in mouse spleen (Amu et al. 2010). These B cells express high levels of CD1d, CD5, CD21, and IgM but low levels of CD23 (Amu et al. 2010), consistent with an innate-like marginal zone or B1 B cell phenotype. Interestingly, mice previously infected with the helminth are less susceptible to de novo or established ovalbumin-induced asthma (Amu et al. 2010), suggesting that the IL10-competent B cells in these mice could contribute to such protection. In fact, helminth-immune mouse B cells, especially $CD1d^{high}$ B cells, can transfer asthma suppression in a B cell-intrinsic IL10-dependent manner (Amu et al. 2010). This transferred suppression correlates with increased numbers of Treg-phenotype cells in the recipient lung, draining lymph node, and airways and, in fact, requires $CD25^+$ recipient cells (Amu et al. 2010). These observations collectively suggest that the Breg-Treg axis could be exploited for immunotherapy against asthma and hypersensitivity.

Additional evidence suggests that Bregs can help control autoimmune disease as well. In the TCR-α-deficient model of intestinal inflammation, B cells delay disease onset (Mizoguchi et al. 2002). This suppressive effect requires B cell expression of CD1d and IL10 (but not MHC II) and correlates with reduced expression of *IL1β* mRNA in the colonic mucosa (Mizoguchi et al. 2002). Paradoxically, IL10-competent B cell frequencies and numbers are increased in other autoimmune-prone mouse strains (Yanaba et al. 2009), and some autoimmune human patients have increased frequencies of IL10-competent B cells in circulation (Iwata et al. 2011). Human systemic lupus erythematosus (SLE) is an example of an autoimmune disease in which some patients show increased IL10 potential in the B cell compartment, most of which is reported to be in $CD5^+$ B cells (Amel Kashipaz et al. 2003). It is unknown whether this correlation reflects pro-inflammatory roles for IL10 or if IL10 is being produced in an attempt to dampen inflammation. Either possibility could be supported by the observations that the frequency of IL10-competent SLE B cells correlates with B cell lymphopenia and B cell-derived IL6 production (Amel Kashipaz et al. 2003). By contrast, $CD24^{high}CD38^{high}$ B cells from SLE patients have poor IL10 induction after CD40 engagement and also fail to limit IFN-γ production by activated CD4 T cells (Blair et al. 2010). Because increased IL10 from total SLE B cells and reduced IL10 from $CD24^{high}CD38^{high}$ SLE B cells were reported in separate studies, it is unknown whether the discrepancy can be explained by a shift in excessive IL10 production from B cells of other phenotypes. Resolving this issue would be highly informative for understanding IL10 in lupus and exploiting its suppressive capacity for therapeutic approaches.

Unlike lupus, total B cells from multiple sclerosis (MS) patients secrete less IL10 after in vitro stimulation through CD40 and the BCR (Duddy et al. 2007). However, after these patients are treated with the immunosuppressant mitoxantrone, their B cells are capable of IL10 production at normal levels (Duddy et al. 2007). This observation strongly suggests that poor IL10 production is an acquired, and not an intrinsic trait of MS B cells. In the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), B cells are required for disease remission (Fillatreau et al. 2002; Wolf et al. 1996). B cell contribution to this remission correlates with suppressed IFN-γ production from antigen-restimulated splenocytes and requires the

B cells to express CD40 and to produce IL10 (Fillatreau et al. 2002). Antigen-reactive B cells in EAE-convalescent mice can produce IL10, and BCR reactivity is important for EAE remission (Fillatreau et al. 2002). These observations provide a very rational motivation for investigating the ability to manipulate Bregs to help induce remission in human MS.

Overall, the suppressive capacity of B cells is only beginning to be understood. Evidence from both human and mouse systems argue that further investigation could advance understanding of such cells in order to exploit their therapeutic potential in the context of cancer, infection, asthma, and autoimmunity. Other evidence indicates suppressive B cells in inhibiting graft rejection in mice (Redfield et al. 2011). Thus, multiple venues of disease can benefit from an understanding of Bregs.

Cross-References

- ▶ CD40
- ▶ CD5
- ▶ Resolution of Inflammation
- ▶ Systemic Lupus Erythematosus, Animal Models
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Systemic Lupus Erythematosus, Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Tregs in the Liver
- ▶ Tumor-Infiltrating T Cells

References

Amel Kashipaz MR, Huggins ML, Lanyon P, Robins A, Powell RJ, Todd I. Assessment of Be1 and Be2 cells in systemic lupus erythematosus indicates elevated interleukin-10 producing $CD5^+$ B cells. Lupus. 2003;12:356–63.

Amu S, Saunders SP, Kronenberg M, Mangan NE, Atzberger A, Fallon PG. Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. J Allergy Clin Immunol. 2010;125(1114–1124):e1118.

Anolik JH, Barnard J, Owen T, Zheng B, Kemshetti S, Looney RJ, Sanz I. Delayed memory B cell recovery in peripheral blood and lymphoid tissue in systemic lupus erythematosus after B cell depletion therapy. Arthritis Rheum. 2007;56:3044–56.

Anolik JH, Looney RJ, Lund FE, Randall TD, Sanz I. Insights into the heterogeneity of human B cells: diverse functions, roles in autoimmunity, and use as therapeutic targets. Immunol Res. 2009;45:144–58.

Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, Mauri C. CD19(+) CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity. 2010;32:129–40.

Bouaziz JD, Calbo S, Maho-Vaillant M, Saussine A, Bagot M, Bensussan A, Musette P. IL-10 produced by activated human B cells regulates CD4(+) T-cell activation in vitro. Eur J Immunol. 2010;40:2686–91.

Duddy M, Niino M, Adatia F, Hebert S, Freedman M, Atkins H, Kim HJ, Bar-Or A. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. J Immunol. 2007;178:6092–9.

Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. Nat Immunol. 2002;3:944–50.

Iwata Y, Matsushita T, Horikawa M, Dilillo DJ, Yanaba K, Venturi GM, Szabolcs PM, Bernstein SH, Magro CM, Williams AD, Hall RP, St Clair EW, Tedder TF. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood. 2011;117:530–41.

Jahrsdorfer B, Blackwell SE, Wooldridge JE, Huang J, Andreski MW, Jacobus LS, Taylor CM, Weiner GJ. B-chronic lymphocytic leukemia cells and other B cells can produce granzyme B and gain cytotoxic potential after interleukin-21-based activation. Blood. 2006;108:2712–9.

Martin F, Kearney JF. Marginal-zone B cells. Nat Rev Immunol. 2002;2:323–35.

Maseda D, Smith SH, DiLillo DJ, Bryant JM, Candando KM, Weaver CT, Tedder TF. Regulatory B10 cells differentiate into antibody-secreting cells after transient IL-10 production in vivo. J Immunol. 2012;188:1036–48.

Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol. 2011;30:221–41.

Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. Immunity. 2002;16:219–30.

Neves P, Lampropoulou V, Calderon-Gomez E, Roch T, Stervbo U, Shen P, Kuhl AA, Loddenkemper C, Haury M, Nedospasov SA, Kaufmann SH, Steinhoff U, Calado DP, Fillatreau S. Signaling via the MyD88 adaptor protein in B cells suppresses protective

immunity during Salmonella typhimurium infection. Immunity. 2010;33:777–90.

Ramgolam VS, Sha Y, Marcus KL, Choudhary N, Troiani L, Chopra M, Markovic-Plese S. B cells as a therapeutic target for IFN-beta in relapsing-remitting multiple sclerosis. J Immunol. 2011;186:4518–26.

Redfield 3rd RR, Rodriguez E, Parsons R, Vivek K, Mustafa MM, Noorchashm H, Naji A. Essential role for B cells in transplantation tolerance. Curr Opin Immunol. 2011;23:685–91.

Tadmor T, Zhang Y, Cho HM, Podack ER, Rosenblatt JD. The absence of B lymphocytes reduces the number and function of T-regulatory cells and enhances the anti-tumor response in a murine tumor model. Cancer Immunol Immunother. 2011;60:609–19.

Wolf SD, Dittel BN, Hardardottir F, Janeway Jr CA. Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. J Exp Med. 1996;184:2271–8.

Yanaba K, Bouaziz JD, Matsushita T, Tsubata T, Tedder TF. The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. J Immunol. 2009;182:7459–72.

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Sarcoidosis

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Synonyms

Noncaseating granulomatosis

Definition

Sarcoidosis, a term derived from the Greek word *sarx* meaning "new flesh," is a disorder of unknown cause manifesting as granulomatous inflammation in affected organs. Any organ system can be affected; tissue lesions are characterized by the presence of granulomas without central necrosis (caseation).

Immunopathogenesis

No single trigger for the development of sarcoidosis has been identified. The inflammatory response in affected organs is characterized by a cell-mediated response and aggregation of CD4+ T lymphocytes. Studies demonstrating high levels of IL-12p40 in serum or BAL fluids derived from patients with active sarcoidosis may reflect enhanced secretion of either IL-12 and/or IL-23. In contrast to the presumed Th1 (or Th17) responses seen in other types of granulomatous inflammation, there is a state of peripheral anergy in sarcoidosis associated with increased circulating CD8+ suppressor T lymphocytes and Th2 cytokines.

Epidemiology

Sarcoidosis presents at any age, but initial manifestations most commonly occur between the second and fifth decades with a slight predominance in females (Costabel and Hunninghake 1999; Hosoda et al. 2002). The reported prevalence is higher in African Americans than in Northern Europeans (ACCESS Research Group 1999); the disorder is perceived to be less common in Asians.

Genetics

A role for genetic factors is supported by the observations of difference disease prevalence among ethnic groups and familial clustering (Sverrild et al. 2008). Genetic linkage analyses combined with sib pair and case–control association studies have identified disease susceptibility loci within the Annexin A11 gene (Hofmann et al. 2008) and BTNL2, a regulator of T cell co-stimulation (Valentonyte et al. 2005). Polymorphisms in CARD15/NO2, the gene coding the recognition protein for bacterial

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peptidoglycans linked to constitutive NFκB activation, and the susceptibility gene for both Blau syndrome and perianal Crohn's disease, are associated with disease severity (Sato et al. 2010).

Clinical Features and Presentation

The respiratory system is thought to be involved at some point in almost all patients with sarcoidosis, but clinical manifestations may occur in any number of different organs with disease also initially becoming manifest as ocular, neurologic, or cutaneous abnormalities. Pulmonary presentations include non-productive cough, with or without dyspnea, in association with symmetric hilar/paratracheal adenopathy and variable degrees of interstitial parenchymal abnormalities on chest imaging. Chronic sinusitis due to involvement of the paranasal sinuses may be a presenting feature.

Cutaneous presentations most commonly include dermal pigmented papules and pannicular inflammation. Biopsy of suspect skin lesions confirms the presence of noncaseating granulomatous inflammation in the dermis. Septal panniculitis with erythema nodosum may be a presenting feature of sarcoidosis but almost always occurs in the context of other apparent disease manifestations. The presentation of fever, erythema nodosum, arthralgia, and symmetric hilar adenopathy (Lofgren's syndrome) is a characteristic acute presentation of sarcoidosis.

Up to 40 % of patients with sarcoidosis have musculoskeletal findings (Thelier et al. 2008; Zisman et al. 2002). Acute arthritis in sarcoidosis most commonly presents as an additive symmetrical oligoarthritis predominantly involving peripheral large joints; symmetrical polyarthritis and asymmetrical transient polyarthralgia have been described less frequently (Awada et al. 2003). The course of acute sarcoid arthropathy is typically benign, with spontaneous resolution of arthritis within a few weeks or months, although relapses may occur. Chronic sarcoid arthritis occurs less frequently but tends to follow a progressive course, causing erosive joint changes and deformity in a limited number of joints. Synovial fluid is inflammatory with lymphocytic predominance and protein elevation; synovial histology may show the typical noncaseating granuloma or nonspecific inflammatory synovitis (Torralba and Quismorio 2003). Axial arthritis is an uncommon manifestation of sarcoidosis, with sacroiliitis reported in 6 % or less of patients (Erb et al. 2005). Periarthritic inflammatory changes in the digits may present as soft-tissue swelling, tenosynovitis, and dactylitis.

Osseous lesions, manifest as punched-out areas of cortical as well as medullary bone, are most often asymptomatic and noted as incidental findings, occurring in 3–13 % of patients (Wilcox et al. 2000). The progression of bone destruction may be complicated by superimposed pathological fracture and/or arthropathy of adjacent joints.

Skeletal muscle involvement is common, occurring in up to 75 % of patients; symptoms are frequently absent but may include myalgia and symmetrical muscle weakness (Zisman et al. 2002; Awada et al. 2003; Le Roux et al. 2007). Nodular tumorous sarcoidosis is characterized by multiple intramuscular nodules that are palpable. Characteristic MRI findings demonstrate well-demarcated nodules with a central star-shaped area of hypointensity surrounded by areas of increased T2 signal; biopsy reveals dense central fibrosis surrounded by lymphocytes and epithelioid noncaseating granulomas with giant cells (Otake and Ishigaki 2001; Otake 1994). Acute sarcoid myositis is rare but most often occurs in the context of acute arthritis or periarthritis. Creatine kinase levels are typically elevated, EMG demonstrates a myopathic pattern, and focal areas of muscle edema may be identified on MRI; muscle biopsy reveals acute inflammation in the muscle fascicle and noncaseating granuloma formation (Otake and Ishigaki 2001; Otake 1994; Moore et al. 2005).

Ocular manifestations may involve the lacrimal glands, thereby giving rise to dry eyes and manifestations of keratoconjunctivitis sicca. Orbital myositis may accompany adnexal gland involvement, resulting in inflammatory pseudotumor of the orbit (Prabhakaran et al. 2007). Uveitis may be

insidious or present acutely involving one or both eyes. A distinguishing feature of sarcoid uveitis is that other ocular structures are commonly involved and may be associated with occult findings on high-resolution chest CT (Clement et al. 2010). The concomitant occurrence of uveitis with parotiditis and facial nerve palsy (Heerfordt's syndrome) is a distinct entity unique to sarcoidosis (James and Sharma 2000).

When present, central and/or peripheral nervous system manifestations are the presenting feature of sarcoidosis in up to half of involved cases (Joseph and Scolding 2009). Disease of the leptomeninges may present as an aseptic meningitis syndrome or as cranial neuropathy. Any of the cranial nerves may be affected but seventh nerve lesions are most frequent, followed by ocular and vestibular nerve complications. Hypothalamic and pituitary lesions may present as endocrine dysfunction. Cortical involvement is most commonly manifest as perivascular gray and white matter lesions, giving rise to a variety of focal deficits; peripheral cranial lesions can mimic a meningioma (Krejchi et al. 1998). Spinal cord myelopathy is often multifocal and may occur at any level. Peripheral neuropathy may occur as a consequence of sarcoid vasculitis or granulomatous inflammation of perineural tissues (Vital et al. 2008).

Myocardial involvement may be manifest as pericarditis, left and/or right heart failure, valvular disease, heart block, or arrhythmia. Delayed enhanced contrast MR imaging with gadolinium provides a sensitive means of detecting myocardial disease in sarcoidosis, 18F-fluorodeoxyglucose PET imaging offers an equally if not greater sensitive alternative for patients unsuitable for MR imaging (Ayyala et al. 2008). Potentially fatal arrhythmias are often refractory to medications and are usually best managed with an implantable defibrillating device (AICD) (Banba et al. 2007).

Vascular involvement with sarcoidosis may involve any caliber vessels resulting in aortitis, necrotizing arteritis of medium caliber vessels, and/or leukocytoclastic vasculitis. Necrotizing sarcoid granulomatosis (NSG) is a variant of sarcoid vasculitis presenting as large nodular lesions in the lung. The radiographic appearance may be similar to that seen in patients with ANCA-associated granulomatosis with polyangiitis, but the predominance of lymphoid aggregates with NSG and a paucity of neutrophils and eosinophils distinguish NSG from lung lesions due to ANCA-associated vasculitis.

Diagnosis

The diagnosis of sarcoidosis is best established by histological confirmation of the presence of noncaseating granulomas in biopsy material obtained from lymphoid, lung, dermal, salivary, lacrimal, or muscle tissue. Biopsy is usually not necessary in patients presenting with Lofgren's syndrome, provided histoloplasmosis is excluded with appropriate serologic studies. Clinical laboratory abnormalities including elevations of angiotensin-converting enzyme (ACE) level are nonspecific, not universally abnormal, and are unreliable for diagnostic purposes or as an indicator of disease activity. MRI is the most sensitive imaging modality used to detect active granulomas in bone, joint, and muscle (Moore et al. 2005; Judson 2008) and may be useful to guide biopsy of appropriate tissue to establish the diagnosis.

Treatment

Systemic corticosteroids are the mainstay of treatment for active sarcoidosis that involves musculoskeletal tissue and visceral organs. To avoid toxicity associated with prolonged corticosteroid use, steroid-sparing immune modulators including methotrexate (MTX), antimalarials, azathioprine, cyclosporine, and mycophenolate mofetil have been used with reported success in limited uncontrolled case series. TNF-α inhibitors (e.g., etanercept, infliximab, adalimumab, golimumab) have been used for patients with refractory or recalcitrant sarcoidosis (Sweiss et al. 2005) with reported success although studies to date do not suggest a consistent beneficial effect.

References

ACCESS Research Group. Design of a case control etiologic study of sarcoidosis (ACCESS). J Clin Epidemiol. 1999;52:1173–86.

Awada H, Abi-Karam G, Fayad F. Musculoskeletal and other extrapulmonary disorders in sarcoidosis. Best Pract Res Clin Rheumatol. 2003;17:971–87.

Ayyala U, Nair A, Padilla M. Cardiac sarcoidosis. Clin Chest Med. 2008;29(3):493–508.

Banba K, Kusano KF, Nakamura K, et al. Relationship between arhythmogenesis and disease activity in cardiac sarcoidosis. Heart Rhythm. 2007;4(10):1292–9.

Clement D, Postma G, Rothova A, Grutters JC, Prokop M, de Jong PA. Intraocular sarcoidosis: association of clinical characteristics of uveitis with positive chest high-resolution computed tomography findings. Br J Ophthalmol. 2010;94(2):219–22.

Costabel U, Hunninghake GW. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and other Granulomatous Disorders. Eur Respir J. 1999;14:735–7.

Erb N, Cushley MJ, Kassimos DG, et al. An assessment of back pain and the prevalence of sacroiliitis in sarcoidosis. Chest. 2005;127:192–6.

Hofmann S, Franke A, Fischer A, Jacobs G, Nothnagel M, Gaede KI, Schürmann M, Müller-Quernheim J, Krawczak M, Rosenstiel P, Schreiber S. Genome-wide association study identifies ANXA11 as a new susceptibility locus for sarcoidosis. Nat Genet. 2008;40:1103–6.

Hosoda Y, Sasagawa S, Yasuda N. Epidemiology of sarcoidosis: new frontiers to explore. Curr Opin Pulm Med. 2002;8:424–8.

James DG, Sharma OP. Parotid gland sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2000;17(1):27–32.

Joseph FG, Scolding NJ. Neurosarcoidosis: a study of 30 new cases. J Neurol Neurosurg Psychiatry. 2009;80(3):297–304.

Judson MA. The diagnosis of sarcoidosis. Clin Chest Med. 2008;29:415–27.

Krejchi D, Caldemeyer KS, Vakili ST, Pritz MB. Meningeal sarcoidosis, pseudo-meningioma, and pachymeningitis of the convexity. J Neuroimaging. 1998;8(3):177–9.

Le Roux K, Streichenberger N, Vial C, Petiot P, Feasson L, Bouhour F, Ninet J, Lachenal F, Broussolle C, Sève P. Granulomatous myositis: a clinical study of thirteen cases. Muscle Nerve. 2007;35:171–7.

Moore SL, Teirstein A, Golimbu C. MRI of sarcoidosis patients with musculoskeletal symptoms. AJR Am J Roentgenol. 2005;185:154–9.

Otake S. Sarcoidosis involving skeletal muscle: imaging findings and relative value of imaging procedures. AJR Am J Roentgenol. 1994;162:369–75.

Otake S, Ishigaki T. Muscular sarcoidosis. Semin Musculoskelet Radiol. 2001;5:167–70.

Prabhakaran VC, Saeed P, Esmaeli B, et al. Orbital and adnexal sarcoidosis. Arch Ophthalmol. 2007;125(12):1657–62.

Sato H, Williams HR, Spagnolo P, Abdallah A, Ahmad T, Orchard TR, Copley SJ, Desai SR, Wells AU, du Bois RM, Welsh KI. CARD15/NOD2 polymorphisms are associated with severe pulmonary sarcoidosis. Eur Respir J. 2010;35(2):324–30.

Sverrild A, Backer V, Kyvik KO, Kaprio J, Milman N, Svendsen CB, Thomsen SF. Heredity in sarcoidosis: a registry-based twin study. Thorax. 2008;63(10):894–6.

Sweiss NJ, Welsch MJ, Curran JJ, Ellman MH. Tumor necrosis factor inhibition as a novel treatment for refractory sarcoidosis. Arthritis Rheum. 2005;53(5):788–91.

Thelier N, et al. Osteoarticular involvement in a series of 100 patients with sarcoidosis referred to rheumatology departments. J Rheumatol. 2008;35:1622–8.

Torralba KD, Quismorio Jr FP. Sarcoid arthritis: a review of clinical features, pathology and therapy. Sarcoidosis Vasc Diffuse Lung Dis. 2003;20:95–103.

Valentonyte R, Hampe J, Huse K, et al. Sarcoidosis is associated with a truncating splice mutation in BTNL2. Nat Genet. 2005;37(4):357–64.

Vital A, Lagueny A, Ferrer X, et al. Sarcoid neuropathy: clinico-pathological study of 4 new cases and review of the literature. Clin Neuropathol. 2008;27(2):96–105.

Wilcox A, Bharadwaj P, Sharma OP. Bone sarcoidosis. Curr Opin Rheumatol. 2000;12:321–30.

Zisman DA, Shorr AF, Lynch III JP. Sarcoidosis involving the musculoskeletal system. Semin Respir Crit Care Med. 2002;23:555–70.

Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations

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Synonyms

Fibrosing disorders; Scleroderma; Systemic sclerosis

Definition

Scleroderma or systemic sclerosis (SSc) is a chronic autoimmune disorder with multi-organ system involvement. It has the highest overall morbidity and mortality of any connective tissue disease, and to date, there is no effective therapy.

Introduction

Scleroderma or systemic sclerosis (SSc) is a chronic fibrosing disorder with widespread systemic involvement and the highest overall morbidity and mortality of any connective tissue disease. The etiology of the disease is unknown; however, it is thought to arise from a combination of factors including autoimmunity, fibroblast proliferation, and endothelial cell dysfunction.

Disease Classification

SSc is heterogeneous and can be divided into various subsets – localized, limited, and diffuse. Localized scleroderma is a cutaneous fibrosing disorder that does not have systemic organ involvement. Patients with localized scleroderma develop skin thickening or hyperpigmentation of the skin in patches, described as morphea. Another form of localized SSc is linear scleroderma or en coupe de sabre, in which the skin is affected in a dermatomal pattern, usually on one side of the body.

The limited and diffuse scleroderma subsets are based on the degree and location of cutaneous involvement. Rarely, SSc without skin involvement (sine scleroderma) can occur, where visceral organs such as the lung, kidney, and heart are involved in the absence of cutaneous disease. In limited SSc, skin fibrosis or thickening is limited to the hands and the area distal to the elbows. Diffuse SSc is defined by skin involvement of the trunk, proximal arms, and legs. The face can be involved in either limited or diffuse disease. Diffuse SSc often has a worse prognosis with a higher rate of interstitial lung disease (ILD) and renal dysfunction, while limited SSc carries a higher rate of pulmonary artery hypertension (PAH) and a lower but still clinically significant risk of ILD than diffuse SSc. The key features of limited and diffuse SSc are outlined in Table 1.

Epidemiology, Genetics, and Environmental Exposures

The reported incidence and prevalence of SSc differs based on the geographic population studied, date of data acquisition, and methodological approaches to disease definition. Overall, the estimated prevalence is 276–658 cases per million with an incidence ranging from 13.9 to 21 cases per million/year in the United States and Canada, with lower rates noted in Europe and the lowest rates in Asian cohorts (Steen et al. 1997; Mayes et al. 2003). SSc occurs more frequently in women (4:1 female to male ratio) and the disease is more severe in African Americans and in those who develop the disease later in life.

While certain familial and ethnic groups may have increased susceptibility to SSc (i.e., Choctaw Native Americans), no clear familial or ethnic predisposition has been identified. The concordance rate for SSc among twins is low at 4.7 %. Specific risk-associated alleles have recently been identified that may predict increased risk for SSc, including HLA-DQA1*0501 in men, and HLA-DRB1*01 and HLA-DRB1*11 (Gorlova et al. 2011; Arnett et al. 2010). Recently, HLA DRB1*0407 and *1304 were found to be independent risk factors for scleroderma renal crisis (Nguyen et al. 2011). Associations of single nucleotide polymorphisms (SNP) with SSc are reported in genes encoding endothelin-1, various growth factors such as transforming growth factor (TGF-β) and connective tissue growth factor (CTGF), and various extracellular matrix proteins. These SNPs are associated with risk for the development of SSc.

Environmental exposures may be linked to the development of scleroderma, including silica, polyvinyl chloride, and organic solvents, though rigorous studies to link these exposures to clinical disease are lacking. In addition, numerous

Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations, Table 1 Clinical features of limited and diffuse SSc

Clinical and Laboratory Features	Limited SSc	Diffuse SSc
Autoantibodies	Anti-centromere	Anti-topoisomerase I (Scl-70), anti-RNA polymerase III
Cutaneous	Skin thickening limited to the distal extremities and face	Skin thickening of extremities, trunk, and face
	Calcinosis cutis	Calcinosis cutis
	Telangiectasias	Rapid progression
Vascular	Raynaud's phenomenon: usually prior to skin involvement and severe	Raynaud's phenomenon: coincides with skin thickening and mild
Cardiac	Rare involvement	Risk for myocarditis, pericardial effusion is highest early in disease onset
Pulmonary	ILD: infrequent, later in course	ILD: frequent, early in course of disease
	PAH: frequent, later in course	PAH: usually in association with ILD
Renal	Renal crisis rare or none	Risk of renal crisis within first 5 years of disease onset
Gastrointestinal	Gastroesophageal reflux, dysphagia	Gastroesophageal reflux, dysphagia
Musculoskeletal	Arthralgias	Arthralgias, muscle weakness, tendon friction rubs

drugs have been implicated in the development of SSc-like fibrosis including bleomycin, radiation, cocaine, and taxol, among others.

Laboratory Features

Antinuclear antibodies (ANAs) are detected in almost all SSc patients. The antibody profile can aid in disease classification. For example, the presence of certain antibodies can predict the clinical subset of scleroderma that may develop; in other instances, these antibodies, in the presence of specific clinical findings such as Raynaud's phenomenon and nailfold capillary changes, can aid in early diagnosis.

Patients with limited SSc typically have the anti-centromere pattern of ANA; these patients are at increased risk for the development of pulmonary hypertension. The development of ILD in this population is less common though not insubstantial, while the risk of renal crisis is low. The anti-topoisomerase antibody (Scl-70) is typically found in patients with diffuse SSc and is more commonly associated with ILD and occasionally with renal crisis. Patients with Scl-70 typically have reduced survival compared to patients with the anti-centromere pattern. A third antibody, RNA polymerase III, has been associated with diffuse skin disease and is associated with a higher risk of renal crisis. Other antibody profiles include anti-PM/Scl, which is associated with scleroderma and polymyositis. The anti-U1-RNP (ribonucleoprotein) antibody is associated with mixed connective tissue disease in which patients may have manifestations of various connective tissue diseases including systemic lupus erythematosus, dermatomyositis, and SSc. Two recently described antibodies, the anti-Th/To and anti-U3 RNP, are both specific for SSc and ILD, but are not commonly measured clinically.

Pathogenesis

The pathogenesis of SSc is multifactorial and incompletely understood. The disease is characterized by immune dysfunction, vasculopathy, and fibrosis. The earliest manifestations of SSc include autoimmune dysfunction, inflammation, and endothelial cell dysfunction affecting the vasculature.

Autoimmunity and Inflammation

The innate and adaptive immune system is activated in SSc. As in other rheumatologic diseases such as SLE, increased expression of interferon-responsive genes, for example, the Type I interferon signature, is also found in SSc (Assassi et al. 2010). Type I interferons are secreted through toll-like receptor (TLR) activation on dendritic cells. TLRs are the most widely studied pathogen recognition receptors of the innate immune system and these receptors initiate the immune response. The ligands for TLRs stimulate dendritic cells to produce IFN-α and IL-6, which, in turn, stimulate pro-fibrotic monocytes/macrophages. In addition, activated T cells are present in affected SSc tissue (Chizzolini and Truchetet 2011). Activated macrophages and T cells demonstrate a Th2 polarized immune response, which induces TGF-β and promotes collagen synthesis and a pro-fibrotic response.

Vasculopathy

Vascular injury is one of the initial insults in SSc pathogenesis, as evidenced by the early manifestation of Raynaud's phenomenon in SSc patients. The vasculopathy is associated with alteration in the autonomic and peripheral nervous systems with decreased vasodilatory neuropeptides and increased reactivity of smooth muscle adrenoreceptors (α2), resulting in vasoconstriction. Subsequent endothelial injury leads to dysregulated production of endothelium-derived, vasodilatory (nitric oxide), and vasoconstrictive (endothelin-1) substances. The vasculopathy affects all blood vessels including capillaries, arterioles, and large blood vessels, and ultimately impairs blood flow and tissue oxygenation. The persistent vessel and endothelial cell damage results in intimal hypertrophy and endothelial cell apoptosis with ultimate obliterative vasculopathy, the hallmark of SSc vascular damage.

Fibrosis

Fibrosis is the differentiating factor of scleroderma and sets it apart from other connective tissue diseases. Fibrosis characteristically follows the autoimmune and vascular changes that occur early in SSc and is the result of dysregulated synthesis and accumulation of collagen and extracellular matrix components. Fibroblasts are the main cell type implicated in fibrosis. Over time these fibroblasts acquire features of smooth muscle cells and become myofibroblasts. Myofibroblasts contribute to fibrosis through collagen accumulation and TGF-β production.

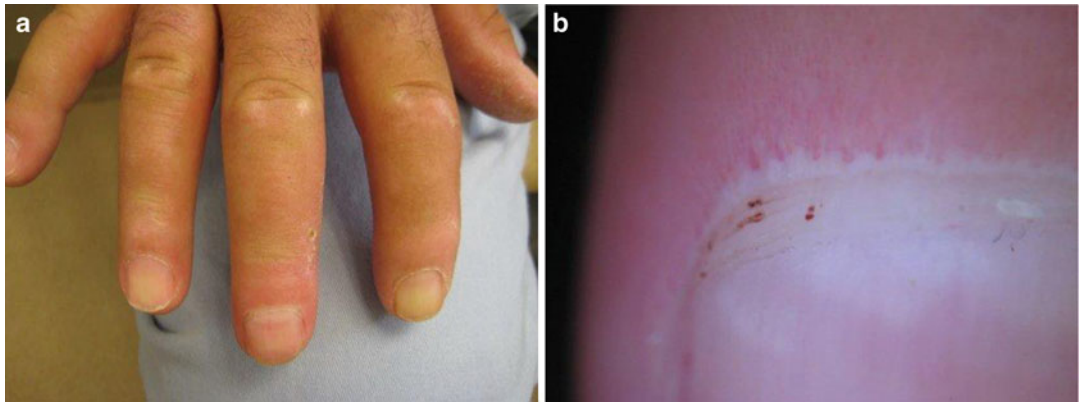
Among the pro-fibrotic cytokines, TGF-β is the central player orchestrating fibrosis. Other key players include CTGF and platelet-derived growth factor (PDGF). The key pathways through which these factors augment the fibroblast response and perpetuate fibrosis are still incompletely understood.

Clinical Features of SSc

Cutaneous Manifestations

Early in SSc, the skin develops edematous or inflammatory changes, which manifest as "puffy" digits with shiny skin. Over the course of several months, these edematous changes lead to skin thickening and fibrosis; in the hands, this is described as "sclerodactyly" (Fig. 1a). The skin usually becomes thickened in a distal to proximal manner. In limited SSc, the skin of the face, neck, and extremities (distal to the elbows) is primarily involved. In diffuse SSc, the skin is affected across multiple regions, including the face, proximal and distal extremities, and trunk. The modified Rodnan skin score (MRSS) is typically used to quantify skin thickness in clinical studies. The MRSS measures skin thickness or tightness in 17 separate body areas with a score of "0" indicating normal skin and a score of "3" indicating severe skin thickness.

Other cutaneous manifestations of SSc include telangiectasias or dilated capillaries, which primarily manifest along the face, oral mucosa, chest, nail beds, and palmar surfaces of the hands. Patients with both limited and diffuse SSc can also develop calcinosis or deposits of calcium hydroxyapatite along the palmar aspects of the digits and extensor surfaces of the extremities.



Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations, Fig. 1 (**a**) Sclerodactyly in a patient with limited SSc. Digits are thickened with evidence of early contractures and periungual erythema. (**b**). Nailfold capillary microscopy of a patient with limited SSc reveals capillary dropout with few giant capillary loops (Courtesy: Joseph Merola, M.D.)

These calcium deposits are difficult to treat and can occasionally drain or become infected.

Cardiovascular Manifestations

Raynaud's phenomenon is one of the first vascular manifestations to arise in SSc patients. Raynaud's phenomenon is a triphasic color change in the digits usually triggered by extremes in temperature. While classically described as a triphasic change, most patients do not report all three phases. Raynaud's phenomenon occurs secondary to vasospasm of the digits and results in sequential blanching or whitening of the digits on exposure to cold, followed by blue/purple changes secondary to ischemia, and then hyperemia or redness during reperfusion. In SSc, nail fold capillary microscopy can be helpful to evaluate for capillary dropout or giant capillary loops (Fig. 1b). These findings are usually seen with connective tissue diseases and are absent in patients with primary Raynaud's phenomenon.

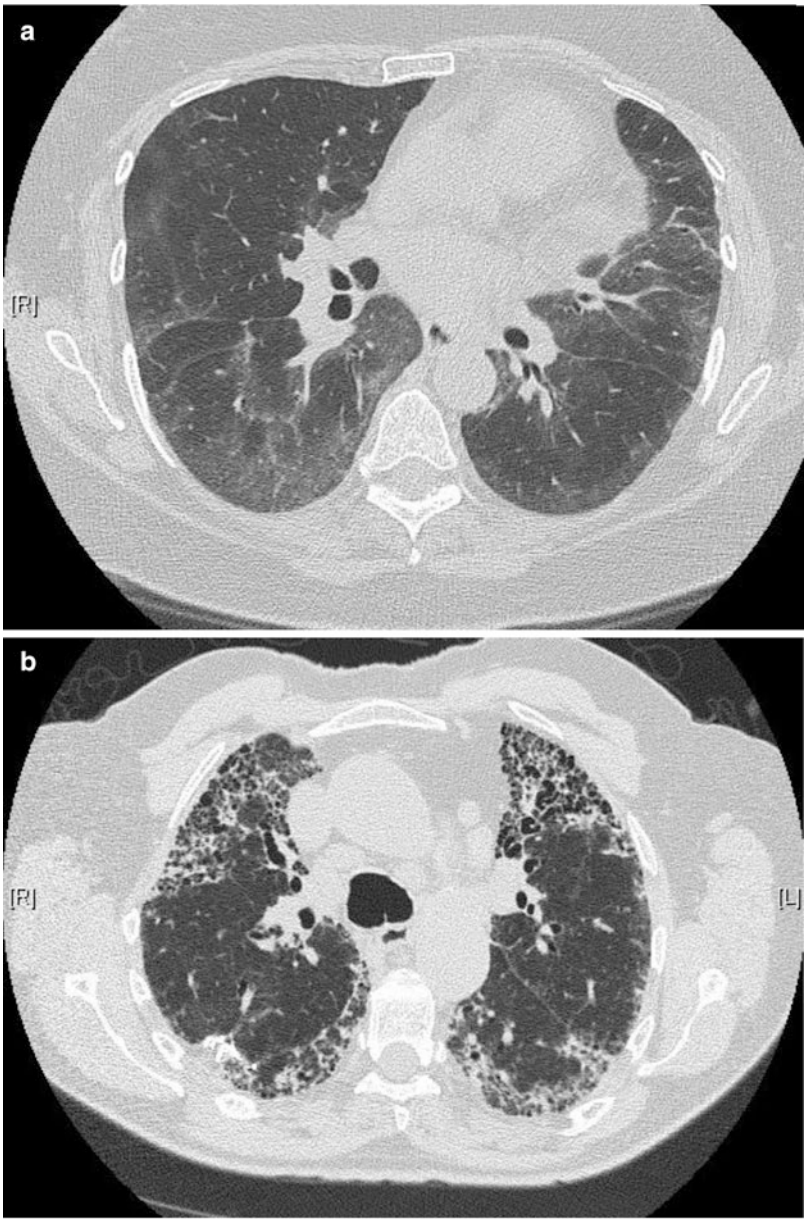
The primary cardiac abnormalities in SSc include contractile dysfunction secondary to myocardial fibrosis, arrhythmias secondary to conduction system fibrosis, coronary artery disease due to microvasculature changes, and pericardial disease (Desai et al. 2011). Additionally, cardiac manifestations can be secondary to pulmonary artery hypertension or interstitial lung disease. Evaluation for cardiac involvement is imperative; echocardiography is a common screening method used to estimate left ventricular function, and assess the pericardium for effusion.

Pulmonary

Interstitial Lung Disease

Pulmonary disease is a leading cause of death in SSc patients. Most patients with SSc have evidence of ILD by high-resolution computed tomography (HRCT) of the chest or at autopsy. Close to half develop clinically significant ILD. In a multi-ethnic study, the risk for ILD in SSc was greater in patients of African-American ethnicity, and those with more extensive skin and cardiac involvement (McNearney et al. 2007). Autoantibody expression is a predictor of internal organ involvement, particularly lung involvement. The presence of anti-topoisomerase antibodies (Scl-70) is strongly associated with development of significant ILD, while anti-centromere antibodies appear to be protective, though patients with limited SSc are not excluded from developing ILD (Kane et al. 1996). In a cohort of 3,656 SSc patients, ILD was present in 53 % with diffuse SSc and in 35 % with limited SSc (Walker et al. 2007).

Histologically, SSc-ILD is characterized by early pulmonary infiltration of inflammatory cells and subsequent fibrosis of the lung



Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations, Fig. 2 (**a**) Nonspecific interstitial pneumonia (*NSIP*) pattern on HRCT. (**b**) Usual interstitial pneumonia (*UIP*) pattern on HRCT with evidence of honeycombing

parenchyma. The most common histologic pattern seen in SSc-ILD is nonspecific interstitial pneumonia (NSIP); usual interstitial pneumonia (UIP)-pattern is less common (Fig. 2). Histologically, NSIP is characterized by varying degrees of inflammation and fibrosis. In contrast, UIP is characterized by dense patchy fibrosis with "honeycombing," primarily in a sub-pleural distribution.

To date, there is no cure or effective disease-modifying therapy for any form of SSc-ILD. Since there is evidence of inflammation in early-stage disease, current therapies for SSc-associated ILD target the inflammatory response. The immunosuppressive agents that are most widely used for this purpose are corticosteroids, cyclophosphamide, azathioprine, and mycophenolate mofetil.

Pulmonary Artery Hypertension

Pulmonary artery hypertension (PAH) can be a primary vasculopathy or secondary to underlying cardiac disease or ILD. PAH is defined as mean pulmonary artery pressure >25 mmHg with a pulmonary capillary wedge pressure <15 mmHg. Patients with PAH often present with dyspnea and fatigue. PAH is predominantly present in patients with limited SSc; however, it can also be present in patients with diffuse SSc and is sometimes secondary to underlying ILD.

Screening for PAH in SSc is recommended and often begins with pulmonary function tests (PFTs) including evaluation of diffusing capacity for carbon monoxide (DLCO) and echocardiography. In isolated PAH, PFTs will demonstrate a reduced DLCO with normal lung function. Echocardiogram can be helpful to approximate right ventricular systolic pressures as an indicator for PAH. If elevated >40 mmHg, right heart catherization is indicated to definitively diagnose PAH. Surrogate markers for PAH have recently been evaluated and serum levels of brain natriuretic peptide (BNP) have been found to correlate with severity of PAH in SSc (Fischer et al. 2012). Therefore, measurements of BNP may be useful for PAH screening in SSc patients.

Gastrointestinal

The gastrointestinal (GI) tract may be involved early in the course of SSc. In 10 % of cases, it is the presenting feature (Clements et al. 2003). The most frequent GI organ involved is the esophagus, followed by the small bowel (Gyger and Baron 2012). The pathogenesis of GI dysfunction stems from vascular changes to the mucosa with neural dysfunction and eventual smooth muscle atrophy.

Abnormal esophageal involvement is the most common GI manifestation in SSc patients with 70–90 % incidence noted depending on the study. Patients have esophageal dysmotility and decreased lower esophageal sphincter tone. While most patients are asymptomatic, therapy with a proton pump inhibitor should be considered for all patients with SSc.

Gastric manifestations of SSc include delayed gastric emptying, which can contribute to esophageal symptoms as well as early satiety and bloating. Other less common manifestations include gastric antral vascular ectasia (GAVE), described as "watermelon stomach," which manifests with severe bleeding. Intestinal dysmotility can lead to bloating, malabsorption, and eventual weight loss and malnutrition. Additionally, bacterial overgrowth of the bowel can result in alternating diarrhea and constipation.

Renal

Scleroderma renal crisis (SRC) is the main renal manifestation and occurs in 10–15 % of patients. It occurs early in the disease course and sometimes precedes the diagnosis of SSc. The classic presentation is new-onset hypertension with progressive renal insufficiency. Risk factors for SRC include diffuse SSc, RNA polymerase III antibody, African-American race, and male gender (Bussone et al. 2011). With the advent of angiotensin-converting enzyme (ACE) inhibitors for the treatment of SRC, the survival from SRC has improved dramatically.

Musculoskeletal

While SSc patients develop myalgias and arthralgias, inflammatory arthritis is less frequent. With progressive skin thickening, patients can develop flexion contractures along the digits, requiring splinting and occupational therapy. Other manifestations include tendon friction rubs, which present along tendon sheaths of the digits, elbows, knees, and ankles. These rubs occur secondary to inflammation and fibrosis of the tendon sheaths at the joint.

Muscle involvement can also occur in patients with SSc and this can be secondary to either deconditioning or malnutrition. SSc patients can additionally have an overlap with polymyositis and test positive for anti-PM/Scl antibody. Occasionally a non-inflammatory myopathy characterized by atrophy and fibrosis in the setting of normal muscle enzymes is seen in the later stages of SSc.

Course and Prognosis

The natural progression of SSc is variable and difficult to predict. Overall, patients with diffuse

disease have rapidly progressive disease with higher mortality, with limited SSc patients progressing slowly. SSc prognosis correlates with skin involvement. Compared to the general population, SSc has a five- to eight-fold higher mortality rate. Major causes of death include PAH, ILD, gastrointestinal involvement, and cardiac disease. While mortality from SRC has been reduced with the advent of ACE inhibitors, the treatment of SSc as a whole is still inadequate and further studies to develop effective therapies are warranted.

Cross-References

- ▶ Fibrosis
- ▶ Raynaud's Phenomenon
- ▶ Scleroderma Renal Crisis
- ▶ Scleroderma: Genetics
- ▶ Scleroderma-Like Conditions of the Skin

References

Arnett FC, Gourh P, Shete S, et al. Major histocompatibility complex (MHC) class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. Ann Rheum Dis. 2010;69(5):822–7.

Assassi S, Mayes MD, et al. Systemic sclerosis and lupus: points in an interferon-mediated continuum. Arthritis Rheum. 2010;62:589–98.

Bussone G, Bérezné A, et al. The scleroderma kidney: progress in risk factors, therapy, and prevention. Curr Rheumatol Rep. 2011;13(1):37–43.

Chizzolini C, Truchetet ME. Fibrosis and immune dysregulation in systemic sclerosis. Autoimmun Rev. 2011;10(5):276–81.

Clements PJ, Becvar R, Drosos AA, Ghattas L, Gabrielli A. Assessment of gastrointestinal involvement. Clin Exp Rheumatol. 2003;21:S15–8.

Desai CS, Lee DC, Shah SJ. Systemic sclerosis and the heart: current diagnosis and management. Curr Opin Rheumatol. 2011;23:545–54.

Fischer A, Bull TM, Steen VD. Practical approach to screening for scleroderma-associated pulmonary arterial hypertension. Arthritis Care Res. 2012;64(3):303–10.

Gorlova O, Marin JE, Rueda B, et al. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. PLoS Genet. 2011;7(7):e1002178.

Gyger G, Baron M. Gastrointestinal manifestations of scleroderma: recent progress in evaluation, pathogenesis, and management. Curr Rheumatol Rep. 2012;14:22–9.

Kane GC, Varga J, Conant EF, Sporn PW, Jimenez S, Fish JE. Lung involvement in systemic sclerosis (scleroderma): relation to classification based on extent of skin involvement or autoantibody status. Respir Med. 1996;90:223–30.

Mayes MD, Lacey JJ, Beebe-Dimmer J, et al. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. Arthritis Rheum. 2003;48:2246–55.

McNearney TA, Reveille JD, Fischbach M, et al. Pulmonary involvement in systemic sclerosis: associations with genetic, serologic, sociodemographic, and behavioral factors. Arthritis Rheum. 2007;57:318–26.

Nguyen B, Mayes MD, Arnett FC, et al. HLA DRB1 *0407 and *1304 are risk factors for scleroderma renal crisis. Arthritis Rheum. 2011;63(2):530–4.

Steen VD, Oddis CV, Conte CG, et al. Incidence of Systemic Sclerosis in Allegheny County. A twenty-year study of hospital diagnosed cases, 1963–1982. Arthritis Rheum. 1997;40:441–51.

Walker UA, Tyndall A, Czirjak L, et al. Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials And Research group database. Ann Rheum Dis. 2007;66:754–63.

Scleroderma Renal Crisis

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Definition

Scleroderma renal crisis (SRC) is a serious complication of systemic sclerosis. It is defined by malignant hypertension and acute kidney failure from prolonged renal ischemia. It is a medical emergency that if left untreated is life threatening and may lead to end-stage renal disease.

Introduction

Systemic sclerosis is a multisystem autoimmune disorder characterized by inflammation, skin

fibrosis, and vascular abnormalities and can manifest as a limited cutaneous or diffuse cutaneous form. Renal failure associated with systemic sclerosis was first described in 1863 (Steen 2003). In 1952 Moore and Sheehan described the renal lesion that was associated with significant morality in scleroderma (see below). SRC is a severe and significant complication of systemic sclerosis that is classically characterized by accelerated hypertension and acute kidney injury. It is prevalent in about 5–10 % of cases and predominantly affects women. SRC is most common with diffuse cutaneous disease but can occur in the limited form. Prior to the advent of angiotensin-converting enzyme inhibitors (ACEi), SRC was almost uniformly fatal. It remains an emergency and has a 5-year survival rate of 65 % (Bussone et al. 2011).

Pathogenesis

The proposed mechanism of SRC begins with endothelial injury followed by increased endothelial permeability and edema. Factors contributing to endothelial injury include vascular collagen accumulation, anti-endothelial cell antibodies, and overexpression of endothelin-1, a potent vasoconstrictor (Worda et al. 2003). This exposes the subendothelium to blood elements activating the coagulation cascade and causing thrombotic proliferative endarteropathy (onion-skin type lesion) (Batal et al. 2010). The renal ischemia that follows causes juxtaglomerular apparatus (JGA) hyperplasia and increased renin secretion leading to accelerated hypertension and kidney injury (Trostle et al. 1988). The end result is thrombotic microangiopathy with renal cortical necrosis.

Renal Pathology

While renal biopsy will confirm the diagnosis of SRC, it is often unnecessary as clinical manifestations are characteristic. Biopsy, however, may be helpful in determining prognosis. On renal biopsy, small-vessel thrombosis is more common than glomerular thrombotic microangiopathy classically seen in TTP/HUS. Prolonged renal ischemia due to vascular disease can lead to ischemic acute tubular necrosis, and if the disease remains unchecked, tubular atrophy and interstitial fibrosis will develop. In untreated disease, duplication of glomerular basement membrane, glomerulosclerosis, and ischemic glomerular collapse may occur (Batal et al. 2009). Severity of acute vascular injury is most predictive of poor outcome, but severe glomerular ischemic collapse and acute tubular necrosis also portend a poorer prognosis.

Clinical Features and Risk Factors

The clinical features of SRC are malignant hypertension and oliguric acute kidney failure. Urine evaluation typically shows microscopic hematuria and subnephrotic proteinuria (<3.5 g/day). Microangiopathic hemolytic anemia has been described in 50 % of cases and leads to abrupt onset of anemia with the presence of schistocytes on peripheral smear and associated thrombocytopenia. Platelet count rarely decreases to less than 50,000/mm^3 and recovers quickly with blood pressure control (Steen 2003). This process is not specific to SRC, and alternative diagnoses, such as disseminated intravascular coagulation (DIC) or thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), must be ruled out. Other clinical findings include headache, blurry vision, and seizures related to hypertensive encephalopathy. Congestive heart failure related to the rapid rise in blood pressure is also possible.

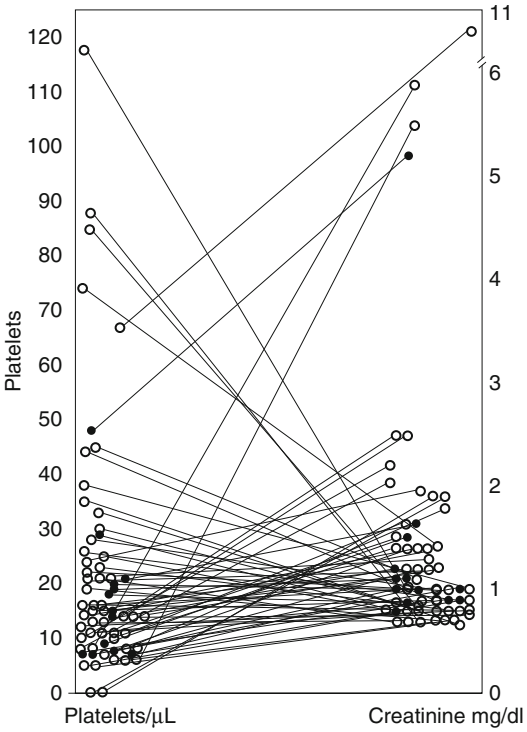
Patients with diffuse cutaneous disease and rapidly progressive skin thickening are at higher risk for SRC and should be monitored closely. In addition conditions that decrease renal perfusion such as new cardiac events or anemia are associated with increased risk for SRC (Steen 2003). Drugs such as systemic corticosteroids, cyclosporine, and cocaine have been associated with SRC. Cyclosporine is thus not recommended after renal transplantation in systemic sclerosis (Bussone et al. 2011). Corticosteroids are thought to inhibit prostacyclin activity and increase tissue ACE activity and may be the mechanism for

increased risk (Steen 2003). Male sex, older age, and *normotension* at diagnosis are thought to be poor prognostic factors (Penn et al. 2007). While vascular changes and hyperreninemia are common in SRC, they do not predict development of SRC and are present in asymptomatic individuals (Trostle et al. 1988). Antinuclear antibodies (ANA) are commonly found in SRC but are nonspecific. Type I and III anti-RNA polymerase antibodies are significantly associated with the development of SRC. Anti-Scl-70 and anti-U3 RNP antibodies have also been associated with SRC; however, anti-centromere antibodies which are commonly seen in limited cutaneous scleroderma have not been associated with SRC (Batal et al. 2010).

Treatment and Prognosis

Aggressive blood pressure control is the mainstay of therapy in SRC and has been shown to stabilize or improve renal function in 50–70 % of cases, if started before irreversible vascular damage has occurred. Inhibition of angiotensin II-mediated vasoconstriction with ACEi is the treatment of choice and significantly improves blood pressure in most patients (Fig. 1). When compared to other antihypertensive agents in SRC, ACEi have increased efficacy, better preserved renal function, and significantly improved survival (Zawada et al. 1981; Thurm and Alexander 1984). ACEi must be continued even if renal function deteriorates and should be titrated to maximum dose over 2–3 days with goal blood pressure of 130/80 mmHg. If blood pressure remains uncontrolled after maximum ACEi, then addition of vasodilator agents should be considered. Calcium channel blockers should be combined with ACEi for added benefit. Nitrates may be used for acute pulmonary edema (Denton and Black 2004). Diuretics should be used with caution as patients have relative hypovolemia in SRC.

If ACEi are not tolerated, angiotensin receptor blockers (ARBs) may be used although the clinical experience with these agents compared to ACEi has been variable and should be used with caution. Recently, a small open-label trial of six patients demonstrated improvement similar to that seen with ACEi after 6 months of therapy with the endothelin receptor antagonist bosentan (Dhaun et al. 2009).



Scleroderma Renal Crisis, Fig. 1 Treatment of scleroderma renal crisis (These agents have been used in smaller studies with variable benefit. Larger trials are warranted prior to recommending their use. ET receptor antagonists appear promising and have shown to have benefit in small trials. IV Prostacyclins have been used for SRC in Europe but there are no large randomized clinical trials to date)

Intravenous prostacyclin is thought to mitigate the microvascular lesion without causing hypotension and has been used at the onset of SRC in Europe (Bussone et al. 2011). However, there are no prospective trials demonstrating benefit. Hemodialysis may be a necessary supportive tool in patients with severe renal failure. With treatment, however, there is usually significant improvement in renal function over time and dialysis may be stopped. Kidney transplantation may be considered in patients who remain dialysis dependent (usually after 2 years) (Pham et al. 2005).

Kidney transplantation provides better outcomes compared to ongoing dialysis for end-stage kidney disease due to SRC. Graft survival, however, is reduced compared to the general renal transplant population and is likely due to recurrence of SRC (Pham et al. 2005).

Conclusion

SRC is a significant complication of systemic sclerosis characterized by accelerated hypertension and acute kidney injury. In SRC, endothelial injury causes thrombotic microangiopathy and renal cortical necrosis. It is a medical emergency, and early recognition is imperative. Aggressive treatment with ACEi and additional antihypertensive agents needed to achieve blood pressure control are necessary to preserve renal function and reduce mortality.

Cross-References

- ▶ Proteinuric Kidney Diseases: Importance of Blood Pressure Control
- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Scleroderma-Like Conditions of the Skin

References

Batal I, Domsic RT, et al. Renal biopsy findings predicting outcome in scleroderma renal crisis. Hum Pathol. 2009;40(3):332–40.

Batal I, Domsic RT, et al. Scleroderma renal crisis: a pathology perspective. Int J Rheumatol. 2010;2010:543704.

Bussone G, Berezne A, et al. The scleroderma kidney: progress in risk factors, therapy, and prevention. Curr Rheumatol Rep. 2011;13(1):37–43.

Denton CP, Black CM. Scleroderma – clinical and pathological advances. Best Pract Res Clin Rheumatol. 2004;18(3):271–90.

Dhaun N, MacIntyre IM, et al. Endothelin receptor antagonism and renin inhibition as treatment options for scleroderma kidney. Am J Kidney Dis. 2009;54(4):726–31.

Penn H, Howie AJ, et al. Scleroderma renal crisis: patient characteristics and long-term outcomes. QJM. 2007;100(8):485–94.

Pham PT, Pham PC, et al. Predictors and risk factors for recurrent scleroderma renal crisis in the kidney allograft: case report and review of the literature. Am J Transplant. 2005;5(10):2565–9.

Steen VD. Scleroderma renal crisis. Rheum Dis Clin North Am. 2003;29(2):315–33.

Thurm RH, Alexander JC. Captopril in the treatment of scleroderma renal crisis. Arch Intern Med. 1984;144(4):733–5.

Trostle DC, Bedetti CD, et al. Renal vascular histology and morphometry in systemic sclerosis. A case–control autopsy study. Arthritis Rheum. 1988;31(3):393–400.

Worda M, Sgonc R, et al. In vivo analysis of the apoptosis-inducing effect of anti-endothelial cell antibodies in systemic sclerosis by the chorioallantoic membrane assay. Arthritis Rheum. 2003;48(9):2605–14.

Zawada Jr ET, Clements PJ, et al. Clinical course of patients with scleroderma renal crisis treated with captopril. Nephron. 1981;27(2):74–8.

Scleroderma, Treatment (Current and Upcoming)

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Synonyms

Progressive systemic sclerosis; Scleroderma; Systemic sclerosis

Definition

The treatment of systemic sclerosis, based on likely pathogenic mechanisms, is directed toward immune cell activation, pathways of collagen synthesis, and, in patients with vascular manifestations, vasoreactive pathways.

Background

Scleroderma (systemic sclerosis, SSc) is a complex disease characterized by (a) extensive ▶ fibrosis that affects the skin and internal organs and can lead to serious compromise of organ function, as in lung fibrosis (interstitial lung disease, ILD); (b) vasculopathy of small arteries (microvasculopathy) with vasospastic episodes (Raynaud's phenomenon) and fibrosis that can cause serious manifestations, such as pulmonary arterial hypertension (PAH), scleroderma renal crisis, and skin ulcers; and (c) activation of the immune system (macrophages, T cells, B cells, and eosinophils), with the production of autoantibodies (Sakkas et al. 2006).

Treatments of scleroderma are based on clinical manifestations and are directed at immune cells (T cells, B cells) and fibroblasts. Treatment directed at immune cells (immunotherapy) is based on evidence of the involvement of immune cells in the pathogenesis of the disease (Sakkas et al. 2006, 2012; Trojanowska and Varga 2007; Maurer and Distler 2011). In skin lesions there is evidence of an antigen-driven T cell activation and proliferation. This involves the interaction of T cell antigen receptor (TCR) on T cells with antigen/HLA complex on antigen-presenting cells, as well as the interaction of co-stimulatory molecules. T cells also help B cells in the production of IgG autoantibodies. A number of autoantibodies in SSc are profibrotic. In addition, T cells in scleroderma patients produce profibrotic soluble cytokine products, including interleukin (IL)-4, IL-13, and IL-17. Transforming growth factor-β (TGF-β) expression is also increased.

Much of the knowledge on human SSc comes from studies in mouse models of scleroderma, particularly bleomycin-induced scleroderma, the tight-skin mouse model of scleroderma, and chronic sclerodermatous graft-versus-host disease (GVHD). The latter develops after hematopoietic stem cell transplantation (HSCT) from one strain into another strain of mouse (allogeneic HSCT).

The treatment of serious clinical manifestations of SSc includes potentially toxic medications. Recommendations regarding treatments based on small nonrandomized studies should be regarded as preliminary until are validated by randomized controlled trials (RCTs). Vascular manifestations, such as skin ulcers, PAH, and scleroderma renal crisis, require additional therapeutic approach (Maurer and Distler 2011).

Immunotherapies

Immunotherapy includes general immunosuppression and therapies targeting specific molecules involved in T cell and B cell survival and function (targeted therapies). Immunotherapies tend to be more effective during early-phase disease. This is because the natural history of SSc, at least as is exemplified in the skin, is first infiltration with T cells, macrophages, B cells, mast cells, and eosinophils, then development of fibrosis with gradual disappearance of inflammatory infiltrates and finally dominance of fibrosis (Prescot et al. 1992; Sakkas et al. 2006). This may explain why immunotherapies may be less effective in late-phase disease.

Cyclophosphamide

Cyclophosphamide (CyP) is an alkylating and cytotoxic agent and, therefore, a general immunosuppressive drug. CyP can be administered either by oral route (e.g., 2 mg/Kg/day) or by intravenous infusions every 2–4 weeks for a maximum of 6 months followed by milder forms of immunosuppression, such as methotrexate or azathioprine. CyP has been used to treat SSc-associated interstitial lung disease (SSc-ILD) in randomized clinical trials. It stabilized if not slightly improved pulmonary function as measured by spirometry and diffusion capacity for carbon monoxide (Hoyles et al. 2006; Taskin et al. 2006; Nannini et al. 2008). It also improved skin score but had no efficacy on skin ulcers in an SSc-ILD study. CyP may improve microvascular damage, as seen by capillaroscopy of nail folds. Side effects of CyP include nausea and vomiting, bone marrow suppression, increased risk of infections, hemorrhagic cystitis, and bladder cancer.

Methotrexate

Methotrexate (MTX) inhibits dihydrofolate reductase, an enzyme needed for DNA synthesis.

It also causes extracellular release of adenosine that exerts immunomodulating effects. MTX, given orally or by subcutaneous injection (10–25 mg/week), is generally a standard, first choice of therapy for rheumatoid arthritis. In two small, randomized, controlled studies of early SSc, MTX improved skin score and hand function (van den Hoogen et al. 1996; Pope et al. 2001). Side effects of MTX include bone marrow suppression, gastrointestinal upset, hair loss, and liver fibrosis.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is an inactive prodrug of mycophenolic acid that inhibits inosine monophosphate dehydrogenase and, therefore, the proliferation of both T cells and B cells. It is administered orally (e.g., 500–1,500 mg twice daily). MMF has been evaluated in small retrospective or open-label prospective studies in SSc and SSc-ILD. It improved skin score and stabilized pulmonary function. More importantly, it improved a 5-year survival (Nihtyanova et al. 2007; Liossis et al. 2006; Koutroumpas et al. 2010). Side effects of MMF include diarrhea, increased risk of infections, and bone marrow suppression.

Azathioprine

Azathioprine (AZA) is a purine analog that inhibits DNA synthesis. AZA has been used as maintenance therapy in SSc-ILD after initial therapy with CyP. In a randomized, open-label trial of oral AZA (2.5 mg/Kg/day) versus oral CyP in early diffuse cutaneous SSc, AZA showed no efficacy on skin thickness and pulmonary function (Nadashkevich et al. 2006). Side effects of AZA include bone marrow suppression and gastrointestinal upset.

Cyclosporin

Cyclosporin binds to cytoplasmic protein cyclophilin and inhibits calcineurin. Calcineurin is a calcium-dependent phosphatase that dephosphorylates nuclear factor of activated T cells (NFAT) that translocates to the nucleus and initiates the transcription of IL-2 and the IL-2 receptor. Therefore, cyclosporin inhibits T cell activation and proliferation. Cyclosporin (2.5 mg/Kg/day, orally) improved skin thickness scores and reduced serum levels of IL-6 in case reports and small open-label studies (Clemens et al. 1993; Filaci et al. 1999). Today, it is rarely used in SSc patients because of its renal side effects. Common effects of cyclosporin include hypertension, elevation in serum creatinine, hypertrichosis, and gingival hypertrophy.

Rapamycin

Rapamycin binds to FK-binding protein 12 and inhibits the mammalian target of rapamycin complex (▶ mTORC) 1, a key regulatory kinase of cell metabolism and proliferation. Rapamycin inhibits T cell activation and Th17 cell differentiation, as well as B cell activation. In TSK mice, rapamycin reduced skin fibrosis and serum levels of autoantibodies. In bleomycin-induced SSc model, administration of rapamycin prior to bleomycin reduced skin and lung fibrosis and skin inflammatory cell infiltrates, as well as the skin expression of IL-4, IL-6, IL-17, and TGF-β. Sirolimus and everolimus, mTORC inhibitors, had beneficial effects in chronic sclerodermatous GVHD. In a small randomized pilot study in patients with early diffuse cutaneous SSc, rapamycin improved skin score but was not superior to MTX. There was no efficacy on lung function (Yoshizaki et al. 2010; Jedlickova et al. 2011; Su et al. 2009). Side effects of rapamycin include gastrointestinal upset, bone marrow suppression, increased risk of infections, elevation in serum triglycerides, and interstitial pneumonitis.

Intravenous Immunoglobulin

Intravenous immunoglobulin derives from pooled plasma of thousands of healthy individuals. The mechanism of high-dose intravenous immunoglobulin (IVIg) remains unclear but probably works on several fronts. One proposed mechanism involves anti-idiotype antibodies directed against unique amino acid sequences on the variable region of immunoglobulins and TCRs that recognize antigens (idiotope). All immunoglobulins and TCRs that share an idiotope belong to one idiotype. Thus, IVIg

blocks the binding of serum immunoglobulins, B cell surface immunoglobulins, as well as TCRs on T cell surface to their respective antigens. Other proposed mechanisms include binding of sialylated IgG to dendritic cells and increasing the expression of their inhibitory receptors for IgG (FcγR) and inhibition of complement.

IVIg reduced skin fibrosis and inhibited IL-4 and TGF-β production in TSK mice (Blank et al. 2002). In two small open-label studies in patients with SSc, IVIg reduced histological skin fibrosis and joint pain and improved the hand function (Levy et al. 2004; Nacci et al. 2007).

Autologous Hematopoietic Stem Cell Transplantation

The process of hematopoietic stem cell transplantation (HSCT) eliminates autoreactive T cells and B cells. In autologous HSCT, hematopoietic stem cells (HSCs) are collected from a patient, who then receives strong immunosuppression, including cyclophosphamide, to destroy lymphocytes. Finally, the patient receives his own HSCs to repopulate his bone marrow. HSCT reverses the Th2 cell profile (producing the profibrotic IL-4 and IL-13) in SSc patients into Th1 cell profile (producing the antifibrotic interferon [IFN]γ) that lasts at least 3 years and is associated with skin improvement (Tsukamoto et al. 2011). A high-dose myeloablative regimen was associated with high frequency of early mortality, and therefore, less intense non-myeloablative regimen was used. In addition, myeloablative HSCT showed no superior efficacy compared to non-myeloablative HSCT (Tyndall and Saccardi 2005). In nonrandomized studies, autologous HSCT showed substantial efficacy in up to 90 % of patients improving skin score and histological fibrosis and stabilizing the internal organ function up to 7 years after the transplantation (Nash et al. 2007; Vonk et al. 2008). It may also regenerate capillaries and improve microcirculation, as seen on nail fold capillaroscopy and histochemistry (Fleming et al. 2009; Miniati et al. 2009). In an open-label randomized trial, autologous non-ablative HSCT was superior to monthly pulse CyP, improving skin score and lung function that persisted for up to 2 years (Burt et al. 2011). Side effects include early mortality, increased risk of infections, and the development of new (secondary) autoimmune diseases, such as myasthenia gravis. Secondary autoimmune diseases occur in about 3.9 % of HSCT cases.

Halofuginone

Halofuginone, a plant alkaloid, inhibits nuclear factor κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK) in activated T cells and also Th17 differentiation. It also inhibits fibroblast collagen production, by inhibiting TGF-β signaling. Topical skin application of halofuginone in chronic GVHD, TSK/+ mice, and patients with SSc improved skin thickness (Pines et al. 2003).

Oral Administration of Type I Collagen

Type I collagen is the most abundant collagen in humans. Human type I collagen could be an autoantigen in SSc, activating T cells. For instance, peripheral blood mononuclear cells cocultured with human type I collagen are activated and express more immune-related genes in patients with early- than late-phase diffuse cutaneous SSc (Atamas et al. 2010). The rationale behind oral administration of bovine type I collagen is to induce tolerance to human type I collagen and that was based on the observation that bovine type I collagen reduced T cell activation and T cell reactivity to human type I collagen. In a randomized, double-blind, placebo-controlled trial, oral administration of bovine type I collagen improved skin score in late-phase SSc (Postlethwaite et al. 2008).

Inhibition of T Cells (Anti-CD25 Monoclonal Antibody)

CD25 is a high-affinity IL-2 receptor expressed on activated T cells and T regulatory cells (Tregs). Tregs, a subset of CD4+ T cells, are important in immune homeostasis because they prevent responses to one's own antigens (autoantigens). Basiliximab, an anti-CD25 monoclonal antibody, decreases the expression of CD25. In a small open-label study,

basiliximab-treated patients with SSc experienced significantly reduced skin thickness scores and stabilized lung function (Becker et al. 2011). A concern regarding basiliximab is that it may decrease Tregs, though this was not observed after short-term treatment in patients with kidney transplantation (Wang et al. 2009).

Inhibition of Co-stimulator Molecules

The activation of T cells by an antigen requires a second signal provided by the interaction of co-stimulatory molecules. The interaction of ▶ CD28 (on T cells) with B7(CD80/CD86) (on antigen-presenting cells) is the best studied such a co-stimulatory interaction. A recombinant ▶ CTLA4-Ig fusion protein consisting of the extracellular domain of CTLA4 and the Fc fragment of IgG1 (abatacept) binds to CD80/CD86 (on antigen-presenting cells), thus blocking CD28 binding to CD80/CD86. This block decreases T cell activation. Based on the significant role of T cells in SSc, a trial of abatacept in SSc has been carried out [www.clinicaltrials.gov. NCT00442611].

B Cell Depletion

B cells have CD20 on their surface. Administration of anti-CD20 monoclonal antibody that depletes B cells in TSK/+ mice before the development of the disease significantly reduces skin fibrosis and serum levels of autoantibodies (Hasegawa et al. 2006). It also reduced skin Th2 cells that produce the profibrotic IL-4 and increased Th1 cells that produce the antifibrotic interferon-γ (IFN-γ). However, anti-CD20 monoclonal antibody had no efficacy when administered after disease development in these mice. Rituximab is a chimeric (part human, part murine) IgG monoclonal antibody that binds CD20 and depletes B cells and has been licensed for use in B cell lymphoma and rheumatoid arthritis. In small open-label studies in SSc, Rituximab improved skin thickness score and reduced serum levels of IL-6 (Bosello et al. 2010). It also improved or stabilized lung function and reduced histological skin fibrosis (Daoussis et al. 2010; Smith et al. 2013). Rituximab may decrease skin fibrosis through attenuation of platelet-derived growth factor receptor expression (Daoussis et al. 2012). However, rituximab did not improve skin thickness score in one study (Lafyatis et al. 2009). A randomized controlled trial of rituximab in SSc-associated PAH is ongoing [ClinicalTrials.gov. NCT01086540].

Inhibition of B Cell Survival

B cell activating factor (BAFF) is a B cell modulator and maturation factor and is increased in SSc. Neutralization of BAFF with BAFF receptor-Ig fusion protein in TSK mice inhibited skin fibrosis and autoantibody production (Matsushita et al. 2007). Anti-BAFF monoclonal antibody (belimumab) is being evaluated in systemic lupus erythematosus.

Interferon-γ

Interferon-γ (IFN-γ) is a cytokine produced by Th1 cells. It inhibits collagen production both in vitro and in vivo in animal models. It also inhibits Th2 differentiation. Recombinant IFN-γ had no effect or slightly improved skin thickness score in small, open-label, single-arm (without comparator) studies (Freundlich et al. 1992; Hunzelmann et al. 1997) and had a mild beneficial effect on skin score in a small randomized controlled trial (Grassegger et al. 1998).

Inhibition of IL-13

Interleukin-13 (IL-13) is a profibrotic cytokine produced by Th2 cells. Inhibition of IL-13-receptor α2 (IL-13Rα2) signaling in bleomycin-induced lung fibrosis with IL-13Rα2-specific small interfering RNA (siRNA) markedly reduced lung fibrosis (Fichtner-Feigl et al. 2006). There are two trials ongoing in SSc-ILD and idiopathic pulmonary fibrosis with anti-IL-13 monoclonal antibody [www.clinicaltrials.gov, NCT00581997, NCT00532233].

Anti-IL-6 Receptor Monoclonal Antibody

IL-6 is a fibroblast growth factor and promotes Th17 differentiation. Treatment of two patients with SSc with anti-IL-6 receptor monoclonal antibody (tocilizumab) decreased skin fibrosis (Shima et al. 2010). A trial of

tocilizumab in SSc is ongoing [clinicaltrials.gov.NCT01532869].

Fibroblast-Directed Therapies

Fibroblasts are the final effector cells in fibrosis. They are activated into myofibroblasts (acquiring α-smooth muscle actin) and produce collagen and other extracellular matrix molecules. Apart from resident fibroblasts, other sources of myofibroblasts are circulating fibrocytes, which are bone marrow-derived mesenchymal cells carrying hematopoietic and stromal cell markers (CD34, CD45 procollagen-I), from pericytes, and from transition of epithelial cells, endothelial cells, and adipocytes into mesenchymal cells, capable of collagen production.

Neutralization of Transforming Growth Factor-β (TGF-β)

Transforming growth factor-β (TGF-β) is a major inducer of fibroblast activation and collagen production. After binding to its receptor, TGF-β activates Smad2 and Smad3 which then form heterotrimer with Smad4 that activates the transcription of target genes. More importantly, TGF-β is involved in the differentiation of epithelial cells, endothelial cells, and adipocytes into mesenchymal cells (transition). This transition involves the acquisition of stromal cell markers and the ability of collagen production (Trojanowska and Varga 2007). An anti-TGF-β polyclonal antibody prevented skin and lung fibrosis in sclerodermatous GVHD mouse model (McCormick et al. 1999). However, a recombinant human anti-TGFβ1 monoclonal antibody was not superior to placebo in a randomized controlled trial (Denton et al. 2007).

Tyrosine Kinase Inhibitors

Imatinib mesylate is a tyrosine kinase inhibitor of c-abl and of proto-oncogene c-kit and c-fms proteins and also of platelet-derived growth factor (PDGF) receptor. c-abl is a downstream mediator of TGF-β. Imatinib reduced fibroblast synthesis of collagen in culture, prevented skin thickness in TSK-1 mice, and reduced bleomycin-induced established skin fibrosis (Akhmetshima et al. 2009). Imatinib also modulates Th2 cytokines in vivo. In SSc-associated ILD, imatinib greatly reduced IL-4-producing T cells in bronchoalveolar lavage (Divekar et al. 2011).

In a patient with SSc, imatinib improved skin fibrosis. In a patient with SSc-associated PAH and right heart failure refractory to bosentan and sildenafil, the addition of imatinib significantly improved right ventricular function and exercise tolerance (Sfikakis et al. 2008; ten Freyhaus et al. 2009). Imatinib at a dose of 400 mg daily was not tolerated in a small, randomized, 6-month trial. In a 1-year, single-arm, open-label phase II trial, imatinib (400 mg/day) was tolerated by most patients and improved skin thickening and forced vital capacity. In another 1-year, phase I/IIa trial in patients with SSc-associated ILD, imatinib (up to 600 mg/day) showed a trend toward improvement of forced vital capacity and a modest improvement in skin score but was associated with frequent side effects. In an observational study of five patients with advanced SSc-associated ILD, a combination of imatinib (200 mg/day) with CyP (500 mg every 3 weeks, intravenously) was well tolerated but improved lung function in only one patient who had mild restrictive lung disease (Pope et al. 2011; Spiera et al. 2011; Khanna et al. 2011; Sabnani et al. 2009). Side effects of imatinib include fluid retention, nausea and vomiting, diarrhea, myalgias, alopecia, rash, and anemia.

Peroxisome Proliferator-Activated Receptor-γ

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a nuclear receptor. Upon binding to its ligand or a synthetic agonist, PPAR-γ activates the transcription of genes involved in lipid and glucose metabolism. PPAR-γ is also a negative regulator of collagen synthesis and thus an attractive target of antifibrotic treatment. PPAR-γ expression is reduced in SSc skin and also reduced by TGF-β in fibroblasts. Fibroblast-specific PPAR-γ deletion in bleomycin-induced SSc mice caused increased skin thickening, whereas synthetic PPAR-γ agonists, such as the synthetic cannabinoid ajulemic acid and the antidiabetic drug rosiglitazone, inhibited fibrosis in bleomycin-induced SSc mouse model (Wu et al. 2009; Gonzalez et al. 2012).

Relaxin

Relaxin is a physiologic protein produced by ovary and placenta during pregnancy and by the prostate gland. Relaxin reduces TGFβ1-induced fibroblast differentiation and collagen production. Recombinant human relaxin prevented bleomycin-induced lung fibrosis in mice (Unemori et al. 1996). In two randomized, double-blind, placebo-controlled trials in SSc, continuous subcutaneous administration of recombinant human relaxin (10 μg/Kg/day or 25 μm/Kg/day) gave conflicting results on skin thickness (Seibold et al. 2000; Khanna et al. 2009). Side effects of relaxin include elevation in serum creatinine and hypertension.

D-Penicillamine

D-penicillamine (DP) is a chelating agent for divalent cations, such as copper, but also inhibits the cross-linking of collagen. In a randomized trial of high-dose (1,000 mg/day) versus low-dose (125 mg/day) DP in diffuse cutaneous SSc of recent onset, there was improvement in skin thickness score in both groups, with no advantage of high dose over the less toxic low dose (Clemens et al. 1999). The authors interpreted these results as negative. However, DP (mean dose 750 mg/day) decreased skin involvement and improved visceral organ involvement in a retrospective study (Derk et al. 2008). Side effects of DP include membranous nephropathy with proteinuria, cytopenias, skin rash, pemphigus vulgaris, myasthenia gravis, and, rarely, Goodpasture's syndrome. Because of serious and frequent side effects and dubious efficacy, DP is rarely used in SSc today.

Therapies for Vascular Manifestations

Pulmonary Arterial Hypertension

The pharmacological management of SSc-associated pulmonary arterial hypertension (SSc-PAH) targets one or more of the three pathogenic pathways, namely, the increased vasoconstrictor endothelin-1 (ET-1) pathway and the decreased vasodilating pathways of prostacyclin and nitric oxide. Epoprostenol, iloprost, and treprostinil are prostacyclin analogs. Bosentan is an inhibitor of both ET-1 type A and ET-1 type B receptors, and ambrisentan and sitaxsentan are ET-1 type A receptor antagonists. Sildenafil (Revatio) and tadalafil inhibit phosphodiesterase 5, an enzyme that rapidly degrades cyclic guanosine monophosphate, a molecule that mediates the vasodilating effects of nitric oxide. All these agents improve exercise capacity, hemodynamics, and quality of life (Launay et al. 2010), and a treatment with two different mode agents modestly increases exercise capacity but does not appear to improve survival (Fox et al. 2011). Therefore, SSc-PAH still remains a condition with poor prognosis despite optimal treatment.

Scleroderma Renal Crisis

▶ Scleroderma renal crisis (SRC) is manifested by severe elevations in blood pressure, elevation in serum creatinine, and fragmented red cells in the peripheral blood. SRC is a serious condition that frequently leads to chronic renal failure. The treatment that changed the prognosis of SRC is based on angiotensin-converting enzyme (ACE) inhibitors (Steen et al. 1990; Penn et al. 2007; Guillevin et al. 2012). ACE converts angiotensin I into angiotensin II (Ang II), a potent vasopressor exerting its effect directly on arteriolar smooth muscle cells. Ang II binds to angiotensin II type 1 (AT1) receptor and activates connective tissue growth factor (CTGF) and collagen production via the Smad pathway and the p38 MAPK pathway. Ang II also affects immune cells. In Ang II-induced hypertension in mice, T cells have a major role in mediating accelerated microvascular thrombosis (Senchekova et al. 2011). Also, in Ang II-induced pulmonary hypertension in mice, there was vascular infiltration of T cells and increased activation of T cells of pulmonary arteries (Vinh et al. 2010). Furthermore, blocking the B7/CD28 co-stimulation with CTLA4-Ig protein construct decreased pulmonary arterial blood pressure and vascular infiltration of T cells and inhibited T cell activation (Vinh et al. 2010). It should be mentioned that stimulatory antibodies against AT1 receptors are detected in sera of patients with SSc (Riemekasten et al. 2011).

ACE inhibitor therapy has greatly improved the survival of patients with SRC. However, up to

44 % of patients may develop end-stage renal failure requiring hemodialysis.

Skin Ulcers

Iloprost, a prostacyclin analog, can be used for severe digital ischemia and dermal ulcers. Iloprost administration by a 6-h intravenous infusion (0.5–2 ng/Kg/min) for 6 sequential days has led to healing of digital ulcers (Wigley et al. 1994). Bosentan, a dual endothelin receptor antagonist, is used for prophylaxis against new ulcer development. In two RCTs, bosentan reduced the occurrence of new digital ulcers by 30–48 % but had no effect on ulcer healing (Korn et al. 2004; Matucci-Cerinic et al. 2011).

Raynaud's Phenomenon

Raynaud's phenomenon (RP) describes vasospastic episodes in which cold exposure or stress triggers a blanching of the digits due to impaired perfusion, then cyanosis due to ischemia, and finally red discoloration due to reperfusion. Treatment with dihydropyridine-type calcium channel antagonists (e.g., nifedipine) results in modest reduction of the frequency and severity of Raynaud's phenomenon (RP) (Thompson et al. 2001). Similar efficacy was demonstrated with angiotensin II receptor type 1 inhibitors (losartan), phosphodiesterase 5 inhibitors (tadalafil), and iloprost, the latter by intravenous infusion. Oral iloprost and bosentan had no efficacy on RP (Wigley et al. 1998; Herrick 2011).

Conclusion

SSc is a complex disease characterized by extensive fibrosis in tissues and blood vessels. The immune system is implicated in fibroblast activation in early-phase disease. In late-phase disease, the persistent activation of fibroblasts is less clear. Apart from general immunosuppression, pathways involved in immune cell activation and fibroblast activation have recently emerged as likely targets of therapy in SSc. In addition, vasoactive pathways may also affect immune function and are therapeutic targets of vascular manifestations of SSc.

Cross-References

- ▶ B7 and CD28 Families
- ▶ CTLA4-Ig
- ▶ Fibrosis
- ▶ Mammalian Target of Rapamycin (mTOR)
- ▶ Scleroderma Renal Crisis
- ▶ TGF-β

References

Akhmetshima A, Venalis P, Dees C, Busch N, Zwerina J, Schett G, et al. Treatment with imatinib prevents fibrosis in different preclinical models of systemic sclerosis and induces regression of established fibrosis. Arthritis Rheum. 2009;60:219–24.

Atamas SP, Luzina IG, Ingels J, Choi J, Wong WK, Furst DE, et al. Stimulation with type I collagen induces changes in gene expression in peripheral blood mononuclear cells from patients with diffuse cutaneous systemic sclerosis. Clin Exp Immunol. 2010;161:426–35.

Becker MO, Bruckner C, Scherer HU, Wassermann N, Humrich JY, Hanitsch LG, et al. The monoclonal anti-CD25 antibody basiliximab for the treatment of progressive systemic sclerosis: an open-label study. Ann Rheum Dis. 2011;70:1340–1.

Blank M, Levy Y, Amital H, Shoenfeld Y, Pines M, Gerina O. The role of intravenous immunoglobulin therapy in mediating skin fibrosis in tight skin mice. Arthritis Rheum. 2002;46:1689–90.

Bosello S, de Santis M, Lama G, Spano C, Angelucci C, Tolusso B, et al. B cell depletion in diffuse cutaneous systemic sclerosis: safety, skin score modification and IL-6 modulation in up to thirty-six months follow-up open-label trial. Arthritis Res Ther. 2010;12:R54.

Burt R, Shah SJ, Dill K, Grant T, Gheorghiade M, Schoeder J, et al. Autologous, non-myeloablative haematopoietic stem-cell transplantation compared with pulse cyclophosphamide per month for systemic sclerosis (ASSIST): an open-label, randomized phase 2 trial. Lancet. 2011;378:498–506.

Clemens PJ, Lanchenbruch PA, Sterz M, Danovitch G, Hawkins R, Ippoliti A, et al. Cyclosporin in systemic sclerosis: results of a forty-eight week open safety study in ten patients. Arthritis Rheum. 1993;36:75–83.

Clemens PJ, Furst DE, Wong WK, Mayes M, White B, Wigley F, et al. High-dose (HI-DPA) vs low-dose (LO-DPA) penicillamine in early diffuse systemic sclerosis (SSc) trial: analysis of a two-year, double-blind, randomized, controlled clinical trial. Arthritis Rheum. 1999;42:1194–203.

Daoussis D, Liossis S-NC, Tsamandas AC, Kalogeropoulou E, Kazantzi A, Sirinian C, et al. Experience with rituximab in scleroderma: results from 1-year proof-of principle study. Rheumatology. 2010;49:271–80.

Daoussis D, Tsamandas AC, Liossis S-N, Antonopoulos I, Karatza E, Yiannopoulos G, et al. B cell depletion therapy in patients with diffuse systemic sclerosis associates with a significant decrease in PDGFR expression and activation in spindle-like cells in the skin. Arthritis Res Ther. 2012;14:R145.

Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, et al. Recombinant human anti-transforming growth factor β1 antibody therapy in systemic sclerosis. A multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis Rheum. 2007;56:323–33.

Derk CT, Huaman G, Jimenez SA. A retrospective randomly selected cohort study of D-penicillamine treatment in rapidly progressive diffuse cutaneous systemic sclerosis of recent onset. Br J Dermatol. 2008;158:1063–8.

Divekar AA, Khanna D, Abtin F, Maranian P, Saggar R, Saggar R, et al. Treatment with imatinib results in reduced IL-4-producing T cells, but increased CD4+ T cells in the bronchoalveolar lavage of patients with systemic sclerosis. Clin Immunol. 2011;141:293–303.

Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat Med. 2006;12:99–106.

Filaci G, Cutolo M, Scudeletti M, Castagneto C, Derchi L, Gianrossi R, et al. Cyclosporin A and iloprost treatment of systemic sclerosis: clinical results and interleukin-6 serum levels after 12 months of therapy. Rheumatology. 1999;38:992–6.

Fleming JN, Nash RA, McLeod DO, Fiorentino DF, Shulman HM, Connolly MK, et al. Capillary regeneration in scleroderma: stem cell therapy reverses phenotype? PLoS One. 2009;3:e1452.

Fox BD, Shimony A, Langleben D. Meta-analysis of monotherapy versus combination therapy for pulmonary arterial hypertension. Am J Cardiol. 2011;108:1177–82.

Freundlich B, Jimenez JA, Steen VD, Medsger Jr TA, Szkolnicki M, Jaffe HS. Treatment of systemic sclerosis with recombinant interferon-gamma. A phase I/II clinical trial. Arthritis Rheum. 1992;35:1134–42.

Gonzalez EG, Selvi E, Balistreri E, Akhmetshina A, Palumbo K, Lorenzini S, et al. Synthetic cannabinoid ajulemic acid exerts potent antifibrotic effects in experimental models of systemic sclerosis. Ann Rheum Dis. 2012;71:1545–51.

Grassegger A, Schuler G, Hesseberer G, Walker-Hantich B, Jabkowski J, Macheiner W, et al. Interferon-gamma in the treatment of systemic sclerosis: a randomized controlled multicenter trial. Br J Dermatol. 1998;139:639–48.

Guillevin L, Berezne A, Seror R, Texeira L, Pourrat L, Mahr A, et al. Scleroderma renal crisis: a retrospective multicentre study on 91 patients and 427 controls. Rheumatology. 2012;51:460–7.

Hasegawa M, Hamaguchi Y, Yanada K, Bouaziz J-D, Uchida J, Fujimoto M, et al. B lymphocyte depletion reduces skin fibrosis and autoimmunity in the tight-skin mouse model of systemic sclerosis. Am J Pathol. 2006;169:954–66.

Herrick AL. Contemporary management of Raynaud's phenomenon and digital ischemic complications. Curr Opin Rheumatol. 2011;23:255–61.

Hoyles RK, Ellis RW, Wellsbury J, Lees B, Newlands P, Gosh NSL, et al. A multicenter, randomized, double-blind, placebo-controlled trial of corticosteroids and intravenous cyclophosphamide followed by azathioprine for the treatment of pulmonary fibrosis in scleroderma. Arthritis Rheum. 2006;54:3962–70.

Hunzelmann N, Anders S, Fierlbeck G, Hermann K, Albrecht M, et al. Systemic scleroderma. Multicenter trial of 1 year with recombinant interferon gamma. Arch Dermatol. 1997;133:609–13.

Jedlickova Z, Burlakova I, Bug G, Baumann H, Schwerdfeger R, Schleuning M. Therapy of chronic sclerodermatous graft-versus-host disease with mammalian target of rapamycin inhibitors. Biol Blood Marrow Transplant. 2011;17:657–63.

Khanna D, Clemens PJ, Furst DE, Korn JH, Ellman M, Rothfield N, et al. Recombinant human relaxin in the treatment of systemic sclerosis with diffuse cutaneous involvement: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 2009;60:1102–11.

Khanna D, Saggar R, Mayes MD, Abtin F, Clemens PJ, Maranian P, et al. A one-year, phase I/IIa, open-label pilot trial of imatinib mesylate in the treatment of systemic sclerosis-associated active interstitial lung disease. Arthritis Rheum. 2011;63:3540–6.

Korn JH, Mayes M, Matucci-Cerinic M, Rainisio M, Pope J, Hachula E, et al. Digital ulcers in systemic sclerosis: prevention by treatment with bosentan, an oral endothelin receptor antagonist. Arthritis Rheum. 2004;50:3985–93.

Koutroumpas A, Ziogas A, Alexiou I, Barouta G, Sakkas LI. Mycophenolate mofetil in systemic sclerosis-associated interstitial lung disease. Clin Rheumatol. 2010;29:1167–8.

Lafyatis R, Kissin E, York M, Farina G, Viger K, Fritzler MJ, et al. B cell depletion with rituximab in patients with diffuse cutaneous systemic sclerosis. Arthritis Rheum. 2009;60:578–83.

Launay D, Sitbon O, Le Pavec J, Savale L, Tcherakian C, Yaici A, et al. Long-term outcome of systemic sclerosis-associated pulmonary arterial hypertension treated with bosentan as first line monotherapy followed or not by the addition of prostanoids or sildenafil. Rheumatology. 2010;49:490–500.

Levy Y, Amital H, Langevitz P, Nacci F, Righi A, Conforti L, et al. Intravenous immunoglobulin modulates cutaneous involvement and reduces skin fibrosis in systemic sclerosis: an open-label study. Arthritis Rheum. 2004;50:1005–6.

Liossis SN, Bounas A, Antonopoulos AP. Mycophenolate mofetil as first line treatment improves clinically evident early scleroderma lung disease. Rheumatology. 2006;45:1005–8.

Matsushita T, Fujimoto M, Hasegawa M, Matsushita Y, Komura K, Ogawa F, et al. BAFF antagonist attenuates the development of skin fibrosis in tight-skin mice. J Invest Dermatol. 2007;127:2772–80.

Matucci-Cerinic M, Denton CP, Furst DE, Mayes DM, Hsu VM, Carpenter P, et al. Bosentan treatment of digital ulcers related to systemic sclerosis: results from the RAPIDS2 randomized, double-blind, placebo-controlled trial. Ann Rheum Dis. 2011; 70:32–8.

Maurer B, Distler O. Emerging targeted therapies in scleroderma lung and skin fibrosis. Best Pract Res Clin Rheumatol. 2011;25:843–58.

McCormick LL, Zhang Y, Tootell E, Gilliam AC. Anti-TGF-β treatment prevents skin and lung fibrosis in murine sclerodermatous graft-versus-host disease: a model for human scleroderma. J Immunol. 1999;163:5693–9.

Miniati I, Guiducci S, Conforti ML, Rogai V, Fiori G, Cinelli M, et al. Autologous stem cell transplantation improves microcirculation in systemic sclerosis. Ann Rheum Dis. 2009;68:94–8.

Nacci F, Righi A, Conforti ML, Miniati I, Fiori G, Martinovic D, et al. Intravenous immunoglobulins improve the function and ameliorate joint involvement in systemic sclerosis: a pilot study. Ann Rheum Dis. 2007;66:977–9.

Nadashkevich O, Davis P, Fritzler M, Kovalenko W. A randomized unblinded trial of cyclophosphamide versus azathioprine in the treatment of systemic sclerosis. Clin Rheumatol. 2006;25:205–12.

Nannini C, West CP, Erwin PJ, Matteson EL. Effects of cyclophosphamide on pulmonary function in patients with scleroderma and interstitial lung disease: a systematic review and meta-analysis of randomized controlled trials and observational prospective cohorts. Arthritis Res Ther. 2008;10:R124.

Nash RA, McSweeney PA, Crofford LJ, Abidi M, Chen C-S, Godwin JD, et al. High-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for severe systemic sclerosis: long-term follow-up of the US multicenter pilot study. Blood. 2007; 110:1388–96.

Nihtyanova SI, Brough GM, Black CM, Denton CP. Mycophenolate mofetil in diffuse cutaneous systemic sclerosis-a retrospective analysis. Rheumatology. 2007;46:442–5.

Penn H, Howie AJ, Kingdom EJ, Bunn CC, Stratton RJ, Black CM, et al. Scleroderma renal crisis: patient characteristics and long-term outcomes. Q J Med. 2007;100:485–94.

Pines M, Snyder D, Yarkoni S, Nagler A. Halofuginone to treat fibrosis in chronic graft-versus-host disease and scleroderma. Biol Blood Marrow Transplant. 2003;9:417–25.

Pope JE, Bellamy N, Seibold JR, Baron M, Ellman M, Carette S, et al. A randomized controlled trial of methotrexate versus placebo in early diffuse scleroderma. Arthritis Rheum. 2001;44:1351–8.

Pope J, McBain D, Petrich L, Watson S, Vanderhoek L, de Leon F, et al. Imatinib in active diffuse cutaneous systemic sclerosis: results of a six month, randomized, double-blind, placebo-controlled, proof-of-concept pilot study at a single center. Arthritis Rheum. 2011;63:3547–51.

Postlethwaite AE, Wong WK, Clemens P, Chatterjee S, Fesser BJ, Kang AH. A multicenter, randomized, double-blind, placebo-controlled trial of oral type I collagen treatment in patients with diffuse cutaneous systemic sclerosis. Arthritis Rheum. 2008;58:1810–22.

Prescot RJ, Freemont AJ, Jones CJ, Hoyland J, Fielding P. Sequential dermal microvascular and perivascular changes in the development of scleroderma. J Pathol. 1992;166:255–63.

Riemekasten G, Philippe A, Nather M, Slowinski T, Muller DN, Heidecke H, et al. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. Ann Rheum Dis. 2011;70:530–6.

Sabnani I, Zucker MJ, Rosenstein ED, Baran DA, Arrovo LH, Tsang P, et al. A novel therapeutic approach to the treatment of scleroderma-associated pulmonary complications: safety and efficacy of combination therapy with imatinib and cyclophosphamide. Rheumatology. 2009;48:49–52.

Sakkas LI, Chikanza I, Platsoucas CD. Mechanisms of disease: the role of immune cells in the pathogenesis of systemic sclerosis. Nat Clin Pract Rheumatol. 2006;2:679–85.

Sakkas LI, Chikanza IC, Platsoucas CD. Systemic sclerosis: from pathogenesis towards targeted immunotherapies. Curr Rheumatol Rev. 2012;8:45–55.

Seibold JR, Korn JH, Simms R, Clemens PJ, Moreland LW, Mayes MD, et al. Recombinant human relaxin in the treatment of scleroderma. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 2000;132:871–9.

Senchekova EY, Russell J, Kurmaeva E, Ostanin D, Granger DN. Role of T lymphocytes in angiotensin-II-mediated microvascular thrombosis. Hypertension. 2011;58:959–65.

Sfikakis PP, Gorgoulis VG, Katsiari CG, Evangelou K, Kostopoulos C, Black CM. Imatinib for the treatment of refractory, diffuse systemic sclerosis. Rheumatology. 2008;47:735–7.

Shima Y, Kuwahara Y, Murota H, Kitaba S, Kawai M, Hirano T, et al. The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. Rheumatology. 2010;49:2408–12.

Smith V, Piette Y, van Praet JT, Decuman S, Deschepper E, Elewaut D, et al. Two-year results of an open-label pilot study of a 2-treatment course with rituximab in patients with early systemic sclerosis with diffuse cutaneous involvement. J Rheumatol. 2013;40(1):52–7.

Spiera RF, Gordon JK, Mersten JN, Magro CM, Mehta M, Wildman HF, et al. Imatinib mesylate (Gleevec) in the treatment of diffuse cutaneous systemic sclerosis:

results of a 1-year, phase IIa, single arm, open-label trial. Ann Rheum Dis. 2011;70:1003–9.
Steen VD, Costantino JP, Shapiro AP, Medsger Jr TA. Outcome of renal crisis in systemic sclerosis: relation to availability of angiotensin converting enzyme (ACE) inhibitors. Ann Intern Med. 1990;113:352–7.
Su TI, Khanna D, Furst DE, Danovitch G, Burger C, Maranian P, et al. Rapamycin versus methotrexate in early diffuse systemic sclerosis: results from a randomized, single-blind, pilot study. Arthritis Rheum. 2009;60:3821–30.
Taskin DP, Elashoff R, Clemens PJ, Goldin J, Roth MD, Furst DE, et al. Cyclophosphamide versus placebo in scleroderma lung disease. New Engl J Med. 2006;354:2655–66.
ten Freyhaus H, Dimitrescu D, Bovenschute H, Erdmann E, Rosenkranz S. Significant improvement of right ventricular function by imatinib mesylate in scleroderma-associated pulmonary arterial hypertension. Clin Res Cardiol. 2009;98:265–7.
Thompson AE, Shea B, Welch D, Pope JE. Calcium-channel blockers for Raynaud's phenomenon in systemic sclerosis. Arthritis Rheum. 2001;44:1841–7.
Trojanowska M, Varga J. Molecular pathways as novel therapeutic targets in systemic sclerosis. Curr Opin Rheumatol. 2007;19:568–73.
Tsukamoto H, Nagafuji K, Horiuchi T, Mitoma H, Niiro H, Arinobu Y, et al. Analysis of immune reconstitution after autologous CD34+ stem/progenitor cell transplantation for systemic sclerosis: predominant reconstitution of Th1 CD4+ T cells. Rheumatology. 2011;50:944–52.
Tyndall A, Saccardi R. Haematopoietic stem cell transplantation in the treatment of severe autoimmune disease: results from phase I/II studies, prospective randomized tribals, and future directions. Clin Exp Immunol. 2005;11:1–9.
Unemori EN, Pickford LB, Salles AL, Piercy CE, Grove BH, Erikson ME, et al. Relaxin induces an extracellular matrix-degrading phenotype in human lung fibroblasts in vitro and inhibits lung fibrosis in a murine model in vivo. J Clin Invest. 1996;98:2739–45.
van den Hoogen FH, Boerbooms AM, Swaak AJ, Rasker JJ, van Lier HJ, van de Putte LB. Comparison of methotrexate with placebo in the treatment of systemic sclerosis: a 24 week randomized double-blind trial, followed by a 24 week observational trial. Br J Rheumatol. 1996;35:364–72.
Vinh A, Chen W, Blinder Y, Weiss D, Taylor WR, Goronzy JJ, et al. Inhibition and genetic ablation of the B7/CD28 costimulatory axis prevents experimental hypertension. Circulation. 2010;122:2529–37.
Vonk MC, Marjanovic Z, van den Hoogen FH, Zohar S, Schattenberg AVM, Fibbe WE, et al. Long-term follow-up results after autologous haematopoietic stem cell transplantation for severe systemic sclerosis. Ann Rheum Dis. 2008;67:98–104.
Wang Z, Shi B-Y, Qian Y-Y, Cai M, Wang Q. Short-term anti-CD25 monoclonal antibody administration down-regulated CD25 expression without eliminating the neogenetic functional regulatory T cells in kidney transplantation. Clin Exp Immunol. 2009; 155:496–503.
Wigley FM, Wise RA, Seibold JR, McCloskey DA, Kujala G, Medsger Jr TA, et al. Intravenous iloprost infusion in patients with Raynaud phenomenon secondary to systemic sclerosis. A multicenter, placebo-controlled, double-blind study. Ann Intern Med. 1994;120:199–206.
Wigley FM, Korn JH, Csuka ME, Medsger Jr TA, Rothfield NF, Ellman M, et al. Oral iloprost treatment in patients with Raynaud's phenomenon secondary to systemic sclerosis. Arthritis Rheum. 1998;41:670–7.
Wu M, Melichian DS, Chang E, Warner-Blankenship M, Ghosh AK, Varga J. Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-γ. Am J Pathol. 2009;174:519–33.
Yoshizaki A, Yanaba K, Yoshizaki A, Iwata Y, Komura K, Ogawa F, et al. Treatment with rapamycin prevents fibrosis in tight-skin and bleomycin-induced mouse models of systemic sclerosis. Arthritis Rheum. 2010;62:2476–87.

Scleroderma: Genetics

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Synonyms

Systemic sclerosis – genetics

Definition

This is a short entry on the recent advances in understanding the genetic basis of systemic sclerosis (scleroderma). The findings of case–control and genome-wide association studies in systemic sclerosis are discussed.

Background

Systemic sclerosis (SSc or scleroderma) is a rare multisystem disease characterized by immune dysregulation, obliterative vasculopathy, and widespread skin and internal organ fibrosis. SSc is associated with high morbidity and mortality. The standardized mortality ratio in patients with SSc is 3.5 (Elhai et al. 2012) which is higher than the mortality observed in patients with other rheumatic diseases, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), or Sjögren's syndrome. The prevalence of SSc ranges from 24 to 44 cases per 100,000 in North America (Bernatsky et al. 2009; Mayes et al. 2003). SSc is subdivided clinically into limited and diffuse subtypes based on the extent of skin disease. It is also characterized by mutually exclusive and highly specific antinuclear autoantibodies that predict various disease manifestations (Steen 2005). Anti-centromere, anti-topoisomerase I, and anti-RNA polymerase III are the most common SSc-related autoantibodies.

The pathogenesis of SSc remains poorly understood. The disease is thought to result from the interplay of genetic and environmental factors. While the environmental triggers are not well studied, there have been significant advances in understanding the genetic basis of SSc in recent years. SSc recurs more commonly in families than expected. DNA samples collected from large numbers of SSc patients through multicenter, international collaborations, as well as advances in high-throughput gene mapping technology and more sophisticated statistical methods have resulted in identification of several robust genetic susceptibility loci in SSc.

Major Histocompatibility Complex (MHC) and SSc Genetics

The three recently completed genome-wide association studies (GWAS) in SSc (Zhou et al. 2009; Radstake et al. 2010; Allanore et al. 2011) have confirmed results of previous case–control studies that the MHC region (especially HLA Class II) represents the most prominent genetic susceptibility locus in SSc (Arnett et al. 2009; Gladman et al. 2005). MHC associations with SSc have been reported in white, Hispanic, African-American, and Asian patients, but the observed effect sizes are relatively weak. Considerably stronger associations have been observed with each of the SSc autoantibody subgroups studied thus far. For example, in a study examining HLA class II in 1,300 SSc cases, the *DRB1*11:04, DQA1*05:01, DQB1*03:01* haplotype and *DQB1* alleles encoding a non-leucine residue at position 26 were risk loci for SSc, while the *DRB1*07:01, DQA1*02:01, DQB1*02:02* haplotype and *DRB1*15:01* haplotype were negatively correlated and possibly "protective" in dominant and recessive models, respectively (Arnett et al. 2009). However, stronger associations were observed with autoantibody subgroups. *DPB1*13:01* was strongly associated with the anti-topoisomerase subgroup of SSc (odds ratio = 14). The anti-centromere positive subgroup was associated with *DQB1*05:01* (OR: 2.6) and *DQB1*26* epialleles while anti-RNA polymerase III positivity was related to *DRB1*04:04 (OR: 5.1), DRB1*11* and *DQB1*03* alleles in white and Hispanic subjects.

HLA associations have been also reported with less common SSc-related autoantibodies. In a study examining 278 African-American patients with SSc, *HLA-DRB1*08:04* was strongly associated with anti-fibrillarin autoantibodies compared to unaffected African-American controls (OR: 11.5) (Sharif et al. 2011). A GWAS follow-up study with 3,175 white SSc patients indicated that *HLA-DQB1*, *HLA-DPA1/B1*, and *NOTCH4* associations with SSc are likely confined to SSc-specific autoantibodies (Gorlova et al. 2011). Recently developed methods for imputation of HLA alleles based on GWAS data and sequencing will enable examination of specific MHC regions in large SSc patient populations in the near future.

Non-MHC SSc Susceptibility Loci

Case–control studies conducted by independent groups have led to the discovery of several robust

Scleroderma: Genetics, Table 1 Selected non-major histocompatibility complex susceptibility genes for systemic sclerosis which were confirmed in at least two independent studies

	Approximate OR	Potential Function
BANK1	0.6–0.8[a]	Lymphocyte activation – B-cells
BLK	1.2–1.5	Lymphocyte activation – B-cells
CD247	0.6–0.8[a]	Lymphocyte activation – T-cells
CD226	1.0–1.2	Lymphocyte activation – T-cells
IRF5	1.2–1.5	Innate immune signaling – Interferon pathway
MIF	1.0–1.2	Adhesion molecule on endothelial cells
PTPN22	1.2–1.5	Lymphocyte activation – T-cells
STAT4	1.2–1.5	Innate immune signaling and lymphocyte activation – T-cells
TNFSF4	1.2–1.5	Lymphocyte activation – T-cells and B-cells
TNIP1	1.2–1.5	Nuclear factor κB pathway

[a]The minor allele is protective.

susceptibility loci in SSc (reviewed in Martin et al. (2012), Mayes (2012)). The majority of these genes such as *IRF5* (Dieude et al. 2009a), *STAT4* (Rueda et al. 2009), *BANK1* (Dieude et al. 2009b), and *BLK* (Gourh et al. 2010) belong to pathways involved in innate and adaptive immunity.

Three recent GWAS studies in SSc have enabled an unbiased examination of the common genetic variants in the entire genome (Zhou et al. 2009; Radstake et al. 2010; Allanore et al. 2011). These studies have confirmed some of the previously reported associations and have led to discovery of novel susceptibility loci. When examined separately, these gene variants have only a small effect on the development of disease (OR < 1.5).

Table 1 summarizes a list of selected non-MHC susceptibility loci that have been confirmed at least by two independent groups. The majority of these loci belong to genes that are involved in immune regulation. For example, *CD247* encodes the T-cell receptor zeta subunit modulating T-cell activation, *IRF5* belongs to a family of transcription factors in the type I interferon pathway, while *TNIP1* is a negative regulator of the nuclear factor κB pathway. The majority of these risk genes are also susceptibility loci for other autoimmune diseases, especially for SLE, suggesting that individuals with SSc may share a common genetic background with several other autoimmune diseases. This supports the concept of quantitative thresholds in immune-cell signaling in which several genetic factors of relatively small effect may cumulatively create a state of susceptibility to autoimmune diseases (reviewed in (Cho and Gregersen 2011)). Self-reactive B- and T-cells are a normal component of the immune system; however, their effect is usually regulated by the appropriate mechanisms in the thymus, bone marrow, or peripheral blood. According to the concept of quantitative threshold, the implicated genetic variations cumulatively impair the necessary biological processes for regulating auto-reactivity, leading to development of autoimmunity. The validity of this concept in SSc is supported by the fact that several SSc genetic susceptibility loci overlap not only with SLE but also with other autoimmune diseases. For example, *STAT4* is also implicated in rheumatoid arthritis and primary biliary cirrhosis.

Several of the confirmed SSc susceptibility loci show a stronger association with its autoantibody or clinical subtypes (limited versus diffuse) than with the overall disease. For example, multiple non-MHC susceptibility genes, such as *BANK1*, *IRF8*, *SOX5*n *and IRF7*, are mainly associated with the SSc-related autoantibodies (e.g., anti-centromere or anti-topoisomerase I) or clinical subtypes of disease (Carmona et al. 2012).

It is important to point out that the majority of identified loci in GWAS studies merely tag the actual causal genetic variants that have yet to be identified by genetic fine-mapping approaches. Furthermore, the GWAS approach does not allow investigation of rare causal variants because the GWAS platforms provide only sufficient coverage (usually >80 %) for common polymorphisms in the human genome.

Genes do not operate in isolation and are usually involved in intertwined biological processes. Therefore, novel mathematical approaches might lead to discovery of gene-gene

interactions that have a significantly higher impact on the development of SSc in comparison to the effect of each susceptibility variant separately. Furthermore, future mechanistic studies are needed to elucidate the cross-talk of the immune susceptibility loci with the other two prominent pathological features of SSc, namely, vasculopathy and fibrosis.

Cross-References

- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Systemic Lupus Erythematosus, Genetics

References

Allanore Y, Saad M, Dieude P, Avouac J, Distler JH, Amouyel P, Matucci-Cerinic M, Riemekasten G, Airo P, Melchers I, et al. Genome-wide scan identifies TNIP1, PSORS1C1, and RHOB as novel risk loci for systemic sclerosis. PLoS Genet. 2011;7(7):e1002091.

Arnett FC, Gourh P, Shete S, Ahn CW, Honey R, Agarwal SK, Tan FK, McNearney T, Fischbach M, Fritzler MJ, et al. Major Histocompatibility Complex (MHC) class II alleles, haplotypes, and epitopes which confer susceptibility or protection in the fibrosing autoimmune disease systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. Ann Rheum Dis. 2010;69(5):822–7.

Bernatsky S, Joseph L, Pineau CA, Belisle P, Hudson M, Clarke AE. Scleroderma prevalence: demographic variations in a population-based sample. Arthritis Rheum. 2009;61(3):400–4.

Carmona FD, Gutala R, Simeon CP, Carreira P, Ortego-Centeno N, Vicente-Rabaneda E, Garcia-Hernandez FJ, de la Garcia PP, Fernandez-Castro M, Martinez-Estupinan L, et al. Novel identification of the IRF7 region as an anticentromere autoantibody propensity locus in systemic sclerosis. Ann Rheum Dis. 2012;71(1):114–9.

Cho JH, Gregersen PK. Genomics and the multifactorial nature of human autoimmune disease. N Engl J Med. 2011;365(17):1612–23.

Dieude P, Guedj M, Wipff J, Avouac J, Fajardy I, Diot E, Granel B, Sibilia J, Cabane J, Mouthon L, et al. Association between the IRF5 rs2004640 functional polymorphism and systemic sclerosis: a new perspective for pulmonary fibrosis. Arthritis Rheum. 2009a;60(1):225–33.

Dieude P, Wipff J, Guedj M, Ruiz B, Melchers I, Hachulla E, Riemekasten G, Diot E, Hunzelmann N, Sibilia J, et al. BANK1 is a genetic risk factor for diffuse cutaneous systemic sclerosis and has additive effects with IRF5 and STAT4. Arthritis Rheum. 2009b; 60(11):3447–54.

Elhai M, Meune C, Avouac J, Kahan A, Allanore Y. Trends in mortality in patients with systemic sclerosis over 40 years: a systematic review and meta-analysis of cohort studies. Rheumatology (Oxford). 2012;51 (6):1017–26.

Gladman DD, Kung TN, Siannis F, Pellett F, Farewell VT, Lee P. HLA markers for susceptibility and expression in scleroderma. J Rheumatol. 2005;32(8):1481–7.

Gorlova O, Martin JE, Rueda B, Koeleman BP, Ying J, Teruel M, az-Gallo LM, Broen JC, Vonk MC, Simeon CP, et al. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. PLoS Genet. 2011;7(7):e1002178.

Gourh P, Agarwal SK, Martin E, Divecha D, Rueda B, Bunting H, Assassi S, Paz G, Shete S, McNearney T, et al. Association of the C8orf13-BLK region with systemic sclerosis in North-American and European populations. J Autoimmun. 2010;34(2):155–62.

Martin JE, Bossini-Castillo L, Martin J. Unraveling the genetic component of systemic sclerosis. Hum Genet. 2012;131(7):1023–37.

Mayes MD. The genetics of scleroderma: looking into the postgenomic era. Curr Opin Rheumatol. 2012;24(6):677–84.

Mayes MD, Lacey Jr JV, Beebe-Dimmer J, Gillespie BW, Cooper B, Laing TJ, Schottenfeld D. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. Arthritis Rheum. 2003;48(8):2246–55.

Radstake TR, Gorlova O, Rueda B, Martin JE, Alizadeh BZ, Palomino-Morales R, Coenen MJ, Vonk MC, Voskuyl AE, Schuerwegh AJ, et al. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. Nat Genet. 2010;42(5):426–9.

Rueda B, Broen J, Simeon C, Hesselstrand R, Diaz B, Suarez H, Ortego-Centeno N, Riemekasten G, Fonollosa V, Vonk MC, et al. The STAT4 gene influences the genetic predisposition to systemic sclerosis phenotype. Hum Mol Genet. 2009;18(11):2071–7.

Sharif R, Fritzler MJ, Mayes MD, Gonzalez EB, McNearney TA, Draeger H, Baron M, Canadian Scleroderma Research Group, Furst DE, Khanna DK, et al. Anti-fibrillarin antibody in African American patients with systemic sclerosis: immunogenetics, clinical features, and survival analysis. J Rheumatol. 2011;38(8):1622–30.

Steen VD. Autoantibodies in systemic sclerosis. Semin Arthritis Rheum. 2005;35(1):35–42.

Zhou X, Lee JE, Arnett FC, Xiong M, Park MY, Yoo YK, Shin ES, Reveille JD, Mayes MD, Kim JH, et al. HLA-DPB1 and DPB2 are genetic loci for systemic sclerosis: a genome-wide association study in Koreans with replication in North Americans. Arthritis Rheum. 2009;60(12):3807–14.

Scleroderma-Like Conditions of the Skin

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Synonyms

CREST; Diffuse fasciitis with eosinophilia; Diffuse systemic scleroderma; Eosinophilic fasciitis; Generalized lichen myxedematosus; Limited scleroderma; Localized scleroderma; Morphea; Nephrogenic fibrosing dermopathy; Nephrogenic systemic fibrosis; Papular mucinosis; Schulman's disease; Schulman's syndrome; Scleroderma and its subtypes: scleroderma; Sclerodermoid lichen myxedematosus; Scleromyxedema: lichen myxedematosus; Scleromyxedema-like illness of renal disease; Skin fibrosis; Skin sclerosis; Systemic scleroderma

Definition

Scleroderma is a systemic disease with the potential for sclerosis of multiple organ systems including the skin, lungs, kidneys, cardiovascular system, and GI tract (Firestein and Kelley 2009). Prior to modern medicine, the most obvious of these manifestations was the skin, and it is therefore named for this manifestation from the Greek (skleros = hard and derma = skin). However, scleroderma is not the only disease entity that can cause sclerosis or the phenotypically similar phenomenon of fibrosis of the skin. The diseases that show these phenotypes are termed "scleroderma-like conditions."

Historical Background

Other conditions that may present with skin stiffening include other autoimmune- or immune-mediated diseases such as overlap syndromes, eosinophilic fasciitis, lichen sclerosis et atrophicus, or graft-versus-host disease; depositional disorders such as lipodermatosclerosis, scleromyxedema, scleroderma, and systemic amyloidosis; reaction to exogenous agents whether occupational, iatrogenic, or a food contaminant (these include such things as aniline-denatured rapeseed oil, L-tryptophan, polyvinyl chloride, bleomycin, and silica); nephrogenic systemic fibrosis, genetic syndromes including certain subtypes of progeria; and stiff skin syndrome itself. This finding therefore has a wide differential. Despite this wide differential, each of these diseases has characteristic features and history, and if the correct information is elicited from the patient, it should therefore be easy to distinguish them. As the treatment and prognosis, and ongoing screening for associated complications, is dramatically different between diagnoses, it is of high importance to know the key differences between these conditions to differentiate them. The different subtypes of "scleroderma," namely, diffuse, localized, and limited scleroderma, are reviewed elsewhere in this volume. This review focuses on briefly reviewing the most common of the scleroderma-like conditions that would enter into the differential and that are most commonly mistaken for scleroderma by nonspecialists. This chapter does not review such things as bleomycin-induced fibrosis or graft-versus-host disease which should be obvious from a simple medical and medication history or localized fibrosing disorders such as scleroderma or lipodermatosclerosis which by distribution are easy to distinguish.

Scleromyxedema

Scleromyxedema is a rare systemic disease which often first presents with the finding of skin stiffening (Rongioletti and Rebora 2001). It may

present as either localized or diffuse forms. In the localized form, it is generally easily differentiated from scleroderma, as instead of skin stiffening, it consists of 1–3 mm asymptomatic or mildly pruritic dome-shaped papules on the skin. These range in color from waxy and skin colored to occasionally mildly erythematous or hyperpigmented. They are most commonly found on the dorsal hands, face, or extensor surfaces of the arms and legs. They can coalesce or occur in parallel ridges. In patients with the diffuse form, despite the more widespread involvement, like the localized form but unlike diffuse scleroderma, the skin is often still mildly papular or cobblestoned. In diffuse scleromyxedema the glabellar and postauricular areas are often targeted and may lead to "leonine facies," where facial ridges and facial folds become accentuated.

Scleromyxedema usually presents between ages 30 and 70. There is no gender or ethnic predilection.

Histology includes deposition of mucin in the dermis between extensive proliferations of plump stellate fibroblasts. Essential parts of work-up include skin biopsy and a serum protein immunoelectrophoresis. Typically patients have a monoclonal paraproteinemia most commonly of the 7S immunoglobulin G (IgG) with lambda light chain type. The pathologic significance, if any, of this is unknown. Less commonly patients may have an associated Waldenström macroglobulinemia or multiple myeloma, but if present this portends a poorer prognosis. Patients with cardiac or pulmonary involvement also have poor prognoses. Lab work-up including thyroid testing is usually otherwise normal unless systemic involvement has begun to affect other organ systems. Work-up is therefore often directed by history and symptoms and suspicion of organ system involvement.

As in scleroderma, in addition to skin stiffening, patients may have multiorgan involvement. Most common is GI dysmotility, but Raynaud's phenomenon and myopathy have also been reported. Neurologic involvement may range from peripheral neuropathy and carpal tunnel to central nervous system involvement resulting encephalopathy and seizures. Mortality may come either from cardiac and pulmonary involvement in the form of pulmonary hypertension, from complications of medications used in treatment, or from underlying malignancy.

Previously melphalan, an alkylating agent, provided the mainstay of treatment, but improvements were usually short lived, and this medication has been associated with significant morbidity and mortality in the form of sepsis and hematologic malignancies (Harris et al. 1979; Helm and Helm 1987). More recently IVIG has been gaining wider acceptance, and there are an increasing number of reports of significant and durable improvements with the use of this medication (Blum et al. 2008). A wide range of other treatments have also been tried, with varying responses including thalidomide (Jacob et al. 2006), which has the side effect of neuropathy which may exacerbate that found in the underlying disease, as well as NBUVB, PUVA, methotrexate, cyclosporine, azathioprine, and mycophenolate mofetil.

Eosinophilic Fasciitis

Originally reported by Schulman in 1974, this entity was described as peripheral eosinophilia with fasciitis leading to deep fibrosis (Shulman 1975). It is distinguished from scleroderma as it often lacks the systemic symptoms of scleroderma, has no Raynaud's or periungual nail fold capillary changes, involves the deep fascia rather than the dermis, and most commonly lacks sclerodactyly as it spares the digits. Classically, the skin is initially edematous but non-pitting, and patients complain of ache. The onset is often rapid, evolving over days to weeks, and in over half of the cases, a history of an immediately preceding strenuous exercise can be elicited. As the disease progresses, the skin becomes increasingly indurated, but due to the distribution of the fibrosis that tends to be deeper, the skin will often appear as a peau d'orange or dimpled appearance and vasculature on the arms and legs appear sunken giving the classic "groove sign."

Involvement is often symmetric and most body areas can be affected, but the face and hands are rarely involved.

EF onset is usually between the second and sixth decade (Lakhanpal et al. 1988). Although rare in pediatrics, when it does occur, it is more common in girls. In adults, in contrast to scleroderma, which like most autoimmune diseases is more common in women, EF occurs more frequently in men (2:1). It shows a predilection for Caucasians. Although EF generally lacks renal, pulmonary, or cardiac involvement, other than in occasional case reports, a symmetric inflammatory arthritis has been reported in about 40 % of cases and carpal tunnellike findings in 10–15 %.

Skin biopsy can be helpful in diagnosis. In early EF skin biopsy shows a mixed cell infiltrate in the deep fascia with lymphocytes, plasma cells, histiocytes, and eosinophils. Vascular cuffing with lymphocytes and plasma cells can also be seen. Of note distribution of the eosinophils in the fascia may be segmental and can sometimes therefore be missed on biopsy. In addition, in later lesions the inflammatory infiltrate is replaced by sclerosis and thickening of fascia and dermis, and biopsies can sometimes be misinterpreted as representing scleroderma or morphea (Barnes et al. 1979).

Sixty to eighty percent of patients have a peripheral blood eosinophilia which often reflects the degree of eosinophilia found in biopsies. The presence of hypergammaglobulinemia is variable occurring in anywhere from 20 % to 60 % of patients (Antic et al. 2006; Bischoff and Derk 2008). When present it is most often a polyclonal increase in immunoglobulin G. Given an association in a subset of patients with hematological malignancy, screening is appropriate and a basic work-up should also include at a minimum CBC and SPEP with immunofixation.

Imaging is rarely necessary, but MRI of involved areas can show fascial thickening, abnormal signal intensity, and contrast enhancement (Moulton et al. 2005).

For most patients the prognosis is good with around 30 % of patients showing spontaneous remission and up to 70 % of EF patients responding to oral corticosteroids. A small subset has therapy unresponsive and progressive disease, and these patients can also have significant morbidity with joint contractures. Due to the limited number of cases, prospective controlled trials comparing the efficacy of different therapeutics do not exist, but a wide range have been tried and reported in case reports to have varying success including hydroxychloroquine, chloroquine, azathioprine, cyclosporine, dapsone, infliximab, tacrolimus, methotrexate, D-penicillamine, griseofulvin, ketotifen, alpha interferon, IVIG, and antitumor necrosis factor (TNF)-alpha agents (Bischoff and Derk 2008; Khanna et al. 2010). Physical therapy, especially in those with contractures, remains an important adjunct to pharmacological intervention.

Nephrogenic Systemic Fibrosis

Nephrogenic systemic fibrosis (NSF) was first observed in 1997, but currently over 335 cases have been reported to the NSF registry (Cowper et al. 2000; Cowper Nephrogenic Systemic Fibrosis 2001–2009). Clinically patients resemble severe EF patients with rapid onset (days to weeks) of non-pitting edema. This most commonly is symmetric and affects the lower extremities followed by the upper extremities. Truncal involvement can occur, although more commonly only in advanced disease. Involvement of the extremities can result in contractures and affect joint flexion and extension. Early on skin is skin colored to mildly erythematous, but as the disease progresses, hyperpigmentation is not uncommon. Early edema evolves into woody induration and patients often complain of itch, burning, and pain.

Despite the clinical similarity to EF, histologically NSF more closely resembles scleromyxedema with spindled cells extending between fat lobules, fibroblast proliferation, thickened collagen bundles, and mucin deposition. Most of the spindle cells are CD34/procollagen dual-positive cells (Kucher et al. 2005).

All patients have some degree of renal dysfunction, and the vast majority has undergone some form of dialysis, although this latter condition is not true of every confirmed patient. Most have also had MRI with a Gadolinium-based contrast agent (GBCA). For this reason GBCA is highly suspected in etiology and can be found in the skin biopsies of many NSF patients (Boyd et al. 2007). This also explains the appearance of this condition in 1997, as this was when these agents first began to find widespread use in the USA. NSF can affect any age but occurs most commonly between 30 and 50 years of age. It shows no predilection by gender or ethnicity. Reports have occurred from North and South America, Europe, Asia, and Australia, but the majority of reports still remain in the United States likely reflecting the higher use of gadolinium contrast studies in that country.

Some, but not all, patients respond to improvement in their renal function, increased dialysis, or renal transplantation. Reports of treatment success with imatinib mesylate are continuing to increase, and this appears at present the most established treatment for this sometimes devastating disease (Kay and High 2008). Second-line treatments and isolated case reports of varying success have also been given with IVIG, extracorporeal photochemotherapy, UVA with or without psoralen, and alpha interferon, but poor success has been reported with methotrexate and cyclophosphamide.

References

Antic M, Lautenschlager S, Itin PH. Eosinophilic fasciitis 30 years after – what do we really know? Report of 11 patients and review of the literature. Dermatology. 2006;213(2):93–101.

Barnes L, et al. Eosinophilic fasciitis. A pathologic study of twenty cases. Am J Pathol. 1979;96(2):493–518.

Bischoff L, Derk CT. Eosinophilic fasciitis: demographics, disease pattern and response to treatment: report of 12 cases and review of the literature. Int J Dermatol. 2008;47(1):29–35.

Blum M, Wigley FM, Hummers LK. Scleromyxedema: a case series highlighting long-term outcomes of treatment with intravenous immunoglobulin (IVIG). Medicine (Baltimore). 2008;87(1):10–20.

Boyd AS, Zic JA, Abraham JL. Gadolinium deposition in nephrogenic fibrosing dermopathy. J Am Acad Dermatol. 2007;56(1):27–30.

Cowper SE. Nephrogenic Systemic Fibrosis [ICNSFR Website]. 2001–2009. Available at http://www.icnsfr.org. Accessed 06/17/12.

Cowper SE, et al. Scleromyxedema-like cutaneous diseases in renal-dialysis patients. Lancet. 2000; 356(9234):1000–1001.

Firestein GS, Kelley WN. Kelley's textbook of rheumatology, vol. 2. Philadelphia: Saunders/Elsevier; 2009.

Harris RB, et al. Treatment of scleromyxedema with melphalan. Arch Dermatol. 1979;115(3):295–299.

Helm F, Helm TN. Iatrogenic myelomonocytic leukemia following melphalan treatment of scleromyxedema. Cutis. 1987;39(3):219–223.

Jacob SE, Fien S, Kerdel FA. Scleromyxedema, a positive effect with thalidomide. Dermatology. 2006; 213(2):150–152.

Kay J, High WA. Imatinib mesylate treatment of nephrogenic systemic fibrosis. Arthritis Rheum. 2008;58(8):2543–2548.

Khanna D, Agrawal H, Clements PJ. Infliximab may be effective in the treatment of steroid-resistant eosinophilic fasciitis: report of three cases. Rheumatology (Oxford). 2010;49(6):1184–1188.

Kucher C, et al. Histopathologic comparison of nephrogenic fibrosing dermopathy and scleromyxedema. J Cutan Pathol. 2005;32(7):484–490.

Lakhanpal S, et al. Eosinophilic fasciitis: clinical spectrum and therapeutic response in 52 cases. Semin Arthritis Rheum. 1988;17(4):221–231.

Moulton SJ, et al. Eosinophilic fasciitis: spectrum of MRI findings. AJR Am J Roentgenol. 2005;184(3):975–978.

Rongioletti F, Rebora A. Updated classification of papular mucinosis, lichen myxedematosus, and scleromyxedema. J Am Acad Dermatol. 2001;44(2):273–281.

Shulman LE. Diffuse fasciitis with eosinophilia: a new syndrome? Trans Assoc Am Physicians. 1975;88:70–86.

SH2 Domain-containing Inositol Phosphatase-1 (SHIP)

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Synonyms

Inositol polyphosphate-5-phosphatase, 145 kDa; Inpp5d; SHIP; SH2 domain-containing inositol phosphatase

Definition

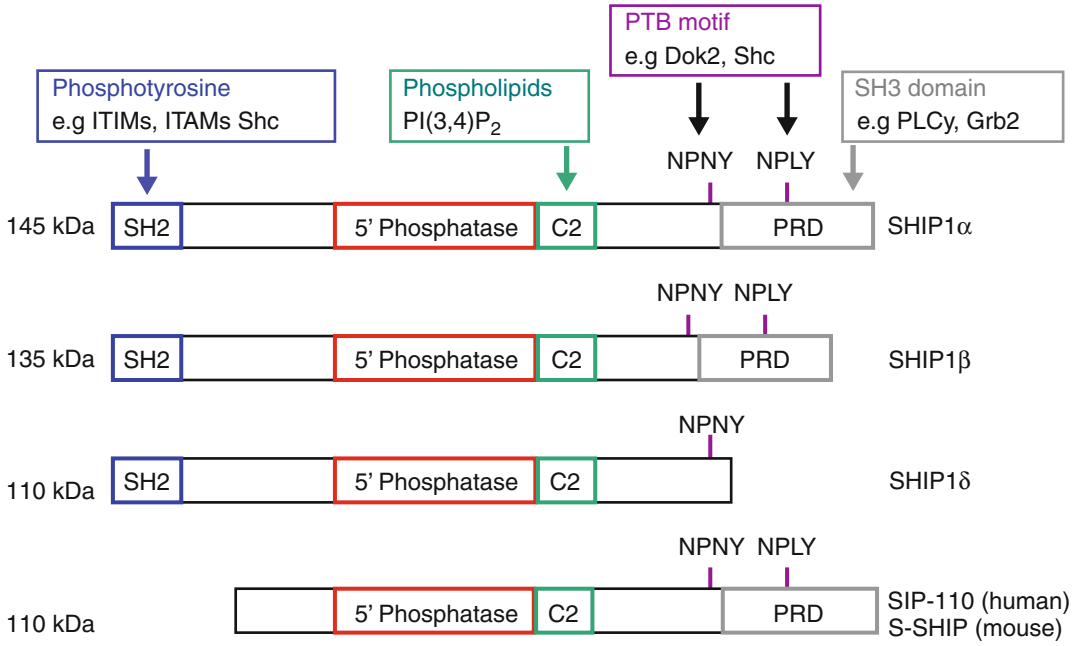
SH2 domain-containing inositol phosphatase-1 (SHIP) is a lipid phosphatase enzyme which regulates phosphoinositide 3-kinase (PI3K) signaling primarily through the degradation of the PI3K product PI(3,4,5)P_3. SHIP is expressed ubiquitously in differentiated cells of the hematopoietic system, in endothelial cells, hemopoietic stem cells (HSC), and embryonic stem (ES) cells. SHIP controls a plethora of immune cell functions and has been the subject of intense research for the past two decades. First identified in 1994, SHIP is a 145 kDa protein which becomes tyrosine phosphorylated after engagement of cell surface receptors on hematopoietic cells (Lioubin et al. 1994; Liu et al. 1994). In the 1990s, the creation of SHIP$^{-/-}$ mice allowed large advances to be made in the understanding of the important role this lipid phosphatase plays in the immune system (Helgason et al. 1998). More detailed analysis followed through the use of cell-restricted deletions of SHIP in myeloid and lymphoid compartments (Leung et al. 2009), while current research is probing further into the role of SHIP in the immune system through the utilization of siRNA and small molecule pharmacological strategies.

Structural Features of SHIP

SHIP protein possesses numerous structural domains as well as a single catalytic domain (Fig. 1). The catalytic domain is responsible for the hydrolysis of the 5-phosphate group on the PI3K product PI(3,4,5)P_3 to form PI(3,4)P_2. Under basal conditions, SHIP is located in the cell cytosol and upon receptor ligation is recruited to the surface membrane, bringing SHIP within close proximity to its lipid substrate. Numerous structural domains are required for SHIP to successfully relocalize to the surface membrane. The SH2 domain within SHIP interacts with proteins via the consensus amino acid sequence pY[Y/S][L/Y/M][L/M/I/V]. Through this SH2 domain, SHIP binds to tyrosine phosphorylated proteins such as Shc, Doks, Gabs, CD150, platelet-endothelial cell adhesion molecule (PECAM), Cas, c-Cbl, certain immunoreceptor tyrosine-based inhibitory motifs (ITIMs), and some immunoreceptor tyrosine-based activation motifs (ITAMs). Proline-rich regions within the C-terminal enable SHIP to bind proteins that contain a SH3 domain, for example, phospholipase-Cγ and Grb2. The phosphorylation of tyrosine residues within the NPXY motifs at the C-terminal tail of SHIP provides sites of interaction for various proteins which express phosphotyrosine-binding (PTB) such as Shc, Dok1, and Dok2. The various structural domains not only serve to bring SHIP in close proximity to its substrate at the surface membrane but also to allow SHIP to perform a scaffolding role, recruiting other proteins to the surface membrane independent of its catalytic activity. SHIP can also negatively regulate PI3K signaling independently of catalytic activity. For example, binding of SHIP to ITAM-containing adaptor proteins via the SH2 domain can prevent PI3K recruitment via the p85 regulatory subunit (Peng et al. 2010).

Variants Within the SHIP Family of Proteins

The *INPP5D* gene located on chromosome 2 (2q37.1) encodes the 145 kDa SHIP protein with multiple forms of SHIP occurring through posttranslational modification, degradation, or alternative mRNA splicing. This produces SHIP proteins of 145 kDa (SHIPα), 135 kDa (SHIPβ), and 110 kDa (SHIPδ) in size. In addition, other 130, 125, and 110 kDa forms of SHIP have been reported (Hamilton et al. 2011; Kerr 2011). Truncated SHIP proteins exhibit differential protein-binding properties owing to the lack of altered expression of certain protein-binding domains (Fig. 1). For example, s-SHIP and its human homologue SIP-110 are truncated at the N-terminus and lack the SH2 domain, but retain the catalytic, C2, and proline-rich domains.



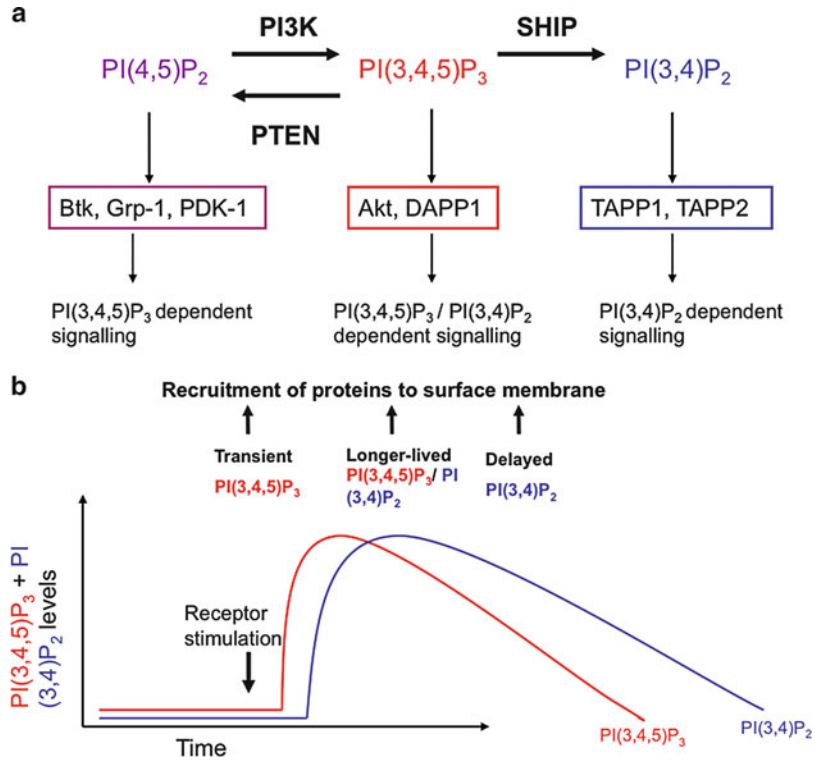
SH2 Domain-containing Inositol Phosphatase-1 (SHIP), Fig. 1 Schematic representation of the SHIP protein isoforms. The protein interaction motifs are indicated, along with their binding partners. The SH2 domain allows SHIP to bind proteins (such as those indicated) which express the sequence pY[Y/S][L/Y/M][L/M/I/V]. The 5′ phosphatase domain catalyzes the conversion of PI (3,4,5)P_3 to PI(3,4)P_2. The C2 domain binds the SHIP product PI(3,4)P_2 which acts to increase the catalytic activity of SHIP. The proline-rich domain (PR) allows SHIP to interact with SH3 domain-containing proteins. The NPXY motifs when phosphorylated provide binding sites for proteins which express phosphotyrosine-binding domains

This limits the repertoire of binding proteins available for interaction, and hence, these forms cannot interact with Shc, yet can still interact with Grb-2. Moreover, s-SHIP is mostly localized at the plasma membrane rather than the cytoplasm (Hamilton et al. 2011; Kerr 2011). Although originally thought to be restricted to embryonic stem cells, s-SHIP expression has been reported in adult hematopoietic cells and synergizes with SHIP to regulate the activation of macrophages (Nguyen et al. 2011).

SHIP-2 is a 142 kDa protein encoded by a separate gene, yet still retains high homology with SHIP. Divergence between SHIP and SHIP-2 occurs within the proline-rich domains and in the ability of SHIP-2 but not SHIP to hydrolyze PI(4,5)P_2 in vitro. SHIP-2 expression is not restricted to hematopoietic cell lineages and can be detected in heart, skeletal muscle, and brain tissues. SHIP-2 appears to have a major role in the negative regulation of insulin signaling in nonimmune cells (Ooms et al. 2009).

SHIP Regulates PI3K Signaling

SHIP degrades the PI3K product PI(3,4,5)P_3 by removing the 5′-phosphate group to yield PI(3,4)P_2. This has lead to the classical view of SHIP acting as a negative regulator of the PI3K/Akt signaling pathway. However, SHIP may also act as a type of molecular switch, able to divert PI3K signaling away from one set of effector proteins toward a second distinct set of effector proteins. Pleckstrin homology (PH) domains encoded in many proteins (e.g., Grp-1) bind exclusively to PI(3,4,5)P_3, whereas others such as that found in dual adaptor of phosphotyrosine and



SH2 Domain-containing Inositol Phosphatase-1 (SHIP), Fig. 2 SHIP acts as a molecular "switch." SHIP catalyzes the conversion of the PI3K lipid product $PI(3,4,5)P_3$ to $PI(3,4)P_2$. Effector proteins which express PH domains are recruited and activated by these lipid second messengers at the cell surface membrane. PH domains of proteins are able to discriminate between $PI(3,4,5)P_3$ and $PI(3,4)P_2$. Examples of proteins which bind only $PI(3,4,5)P_3$, both $PI(3,4,5)P_3$ and $PI(3,4)P_2$, or only $PI(3,4)P_2$ are shown (**a**). This diversity allows SHIP to both negatively regulate PI3K signaling and also to "switch" signal transduction pathways away from $PI(3,4,5)P_3$-dependent effectors toward $PI(3,4)P_2$-dependent effectors, in turn influencing kinetics and duration of recruitment of 3′-phosphoinositide lipid-binding proteins at the plasma membrane (depicted in (**b**))

3-phosphoinositides −1 (DAPP-1) can interact with both $PI(3,4,5)P_3$ and $PI(3,4)P_2$ (Lemmon and Ferguson 2000). In addition, the tandem PH domain-containing protein TAPP-1 encodes PH domains that show exclusive selectivity toward $PI(3,4)P_2$ (Harris et al. 2008). The ability of PH domain-containing proteins to distinguish between different 3′-phosphoinositide lipids suggests that SHIP can redirect PI3K-dependent signaling toward a set of distinct effectors that are temporally and functionally separate from PI $(3,4,5)P_3$-dependent events. Thus, SHIP may function to fine-tune phosphoinositide signaling, rather than terminate it (Fig. 2). In this regard, SHIP promotes recruitment of the GTPase Irgm1 to sites of phagocytosis in macrophages via generation of $PI(3,4)P_2$, a critical step in maturation of the phagosome and engulfment of bacteria. $PI(3,4,5)P_3$ and $PI(3,4)P_2$ appear sequentially following agonist stimulation in many cell types including T lymphocytes but show temporal overlap. Some cell types, notably B lymphocytes and platelets, exhibit sustained PI $(3,4)P_2$ production, lasting for up to 45–60 min poststimulation (Harris et al. 2008).

Role of SHIP in Stem Cell Biology and Transplantation

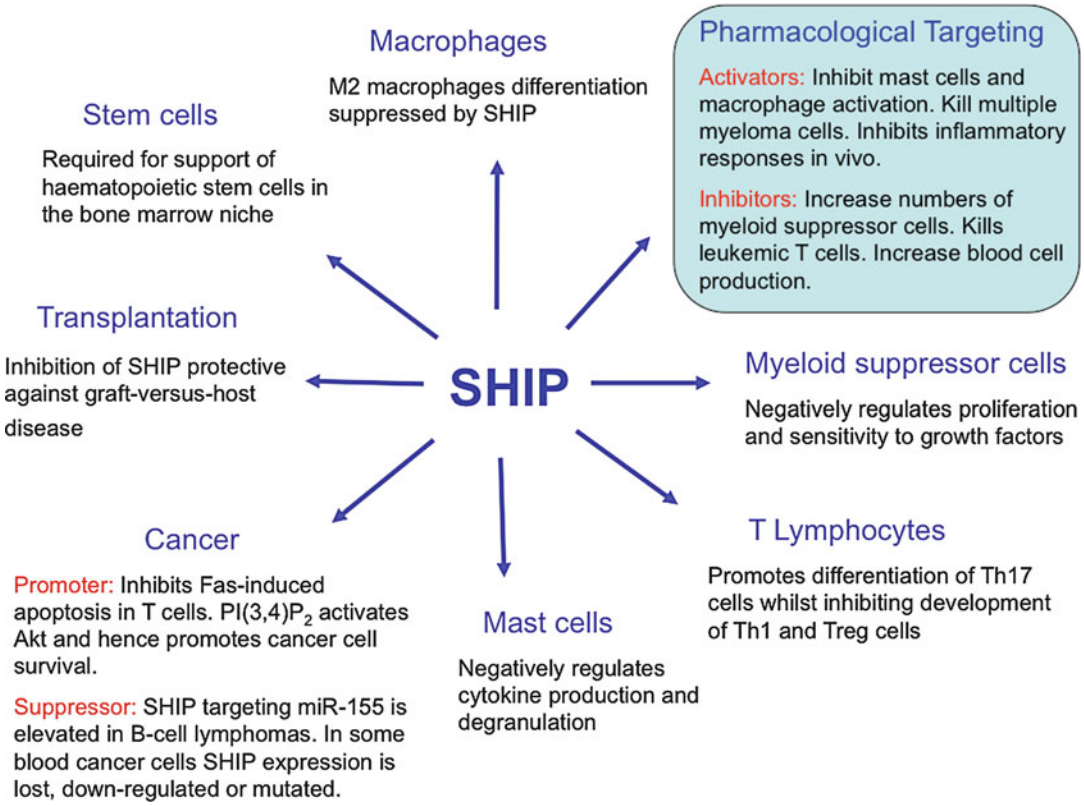
Both SHIP and s-SHIP have been implicated in the biology of pluripotent and adult stem cells.

More recently, it has been shown to play a key role in the function of the bone marrow microenvironment (Kerr 2008). HSC proliferation and numbers are increased in SHIP$^{-/-}$ mice. However, despite expansion of the compartment, SHIP-deficient HSCs exhibit reduced capacity for long-term repopulation and home inefficiently to bone marrow. The role of SHIP in the biology of both HSC and the hematopoietic stem cell niche suggests that it may be a useful target for treatment of bone marrow failure syndromes caused by viruses, radiation, chemotherapy, or malignancy. A common complication arising after bone marrow transplantation is graft-versus-host disease (GVHD) which involves priming of allogeneic T cells. Remarkably, SHIP-deficient mice express more myeloid-derived suppressor cells (MDSCs) than their WT counterparts. MDSCs function to repress allogeneic T cells. Hence, SHIP-deficient mice accept allogeneic bone marrow grafts with a reduced incidence of GVHD (Ghansah et al. 2004; Kerr 2008).

Role of SHIP in the Immune System

SHIP was originally recognized as an important component of the inhibitory signaling pathway triggered by the IgG receptor FcγRIIB in mast cells and B cells (Ono et al. 1996). Once recruited to the plasma membrane by signaling complexes, its catalytic activity depletes PI(3,4,5)P_3 and prevents membrane localization of some PH domain-containing effectors, leading to inhibition of extracellular calcium influx and ultimately reducing transcription activation and cytokine release. SHIP has subsequently also been implicated in signaling pathways triggered by cytokine, chemokine, antigen, and IgG engagement in a variety of immune cells (Harris et al. 2008). SHIP is now known to play a key role in regulating the receptor repertoire and cytolytic function of natural killer (NK) cells, B lymphocyte development and antibody production, the myeloid cell response to bacterial mitogens, development of marginal zone macrophages, lymph node recruitment of dendritic cells, and mast cell degranulation (Fig. 3). The negative regulatory role for SHIP in the immune system is highlighted by the phenotype of SHIP null mice, which develop progressive myeloid hyperplasia and myeloid infiltration in the lungs that leads to respiratory failure and to a dramatic decrease of life span. The myeloid hyperproliferation is caused by the combination of two factors. First, the myeloid cells and their precursors are more sensitive to growth factors, and second, these cells show a decreased sensitivity to proapoptotic stimuli (Helgason et al. 1998). Additionally, SHIP plays an important regulatory role in establishing endotoxin tolerance in macrophages as well as regulating leucocyte polarization during migratory responses to chemoattractants (Hamilton et al. 2011; Harris et al. 2008; Kerr 2011).

SHIP plays a critical role in homeostasis of myeloid and lymphoid effector and regulatory cells. As mentioned above, SHIP-deficient mice exhibit more myeloid-derived suppresser cells (MDSCs) than their wild type counterparts. SHIP also plays a role in regulating the balance of M1 macrophages (implicated in the first inflammatory response) and M2 macrophages (implicated in inflammatory response termination, tissue repair, regeneration, and remodeling). Hence, SHIP deficiency leads to increased macrophage skewing toward M2 macrophages, indicating that PI(3,4,5)P_3 drives macrophage progenitors toward an M2 phenotype which is suppressed by SHIP (Hamilton et al. 2011; Harris et al. 2008; Kerr 2011). SHIP has a key role in regulating CD4$^+$ T cell differentiation via its capacity to promote Th17 and limit regulatory T cell (Treg) development. In vitro and in vivo analyses revealed that in the absence of SHIP, T cells have an enhanced capacity to develop into Tregs with a parallel decrease in Th17 cell development (Hamilton et al. 2011; Harris et al. 2008; Kerr 2011). Curiously, mice with a T cell-specific deletion of SHIP do not skew efficiently to a Th2 phenotype as a result of its inhibitory effect on T-bet expression and consequently display Th1-dominant immune responses in vitro and in vivo (Leung et al. 2009). This is in contrast to evidence from germ line SHIP$^{-/-}$ mice, which



SH2 Domain-containing Inositol Phosphatase-1 (SHIP), Fig. 3 SHIP regulates immune cell functions and tumor development and growth. SHIP plays a key role in the generation of myeloid and lymphocyte subsets, thus maintaining a balance between inflammatory and regulatory cells. Modulation of SHIP offers potential for therapeutic intervention in cancer, transplantation, and a range of inflammatory and autoimmune diseases

indicates that SHIP can also repress Th2 skewing by inhibiting IL-4 production from basophils (Hamilton et al. 2011).

SHIP has been targeted by several opportunistic pathogens in order to evade immune detection. Thus, lymphocytes are particularly sensitive to the cytolethal distending toxin subunit B (CdtB), an immunotoxin produced by *Actinobacillus actinomycetemcomitans*, that can hydrolyze $PI(3,4,5)P_3$ to $PI(3,4)P_2$. Exposure to CdtB leads to cell cycle arrest and death by apoptosis. The lipid phosphatase activity of CdtB may therefore result in reduced immune function, facilitating chronic infection with *Actinobacillus* and other enteropathogens that express Cdt proteins (Shenker et al. 2007). The measles virus induces expression of SIP-110 which depletes the cellular $PI(3,4,5)P_3$ pools, probably leading to increased activation thresholds for T cell activation (Avota et al. 2006).

Role of SHIP in Cancer

PI3K signaling is activated in human cancers via several different mechanisms including activating mutations in the catalytic subunit as well as downregulation of expression of negative regulators such as the 3′ lipid phosphatase PTEN, a well-known tumor suppressor gene (Manning and Cantley 2007). Similarly, SHIP is often lost, downregulated, or mutated in many cancer cells including acute myeloid leukemia (AML) and T acute lymphoblastic leukemia (T-ALL)

(Hamilton et al. 2011). SHIP is also targeted by microRNAs, in particular miR-155. Elevated levels of miR-155 and consequent diminished SHIP expression have been linked to B cell lymphomas (Costinean et al. 2009). In addition, oncogenic proteins such as BCR/Abl and Tax (implicated in chronic myelogenous leukemia and adult T cell leukemia/lymphoma, respectively) can induce SHIP downregulation by a variety of mechanisms (Kerr 2011). Another way in which SHIP can influence tumor growth relates to its role in restricting development of MDSCs and Tregs. Since SHIP deficiency leads to an expansion of MDSCs and Tregs, this could lead to the suppression of antitumor effects mounted by T cell-mediated immune responses. Conversely, there is evidence that SHIP can actually support cancer cell survival. In this regard, SHIP inhibits CD95/APO-1/Fas-induced apoptosis in T cells by promoting CD95 glycosylation independently of its phosphatase activity (Charlier et al. 2010). In addition, pharmacological inhibition of SHIP induces apoptosis of multiple myeloma cells (Brooks et al. 2010). This effect likely arises as a result of the production of $PI(3,4)P_2$ which is known to facilitate Akt activation and, hence, survival and tumorigenesis (Manning and Cantley 2007).

Targeting SHIP with Small Molecules

The role of SHIP in regulating the development and function of various hematopoietic cells and evidence linking SHIP to cancer and other diseases has led to the search for small molecules that are able to modulate activity and which may be useful as drugs. Several compounds that bind to the C2 domain and lead to allosteric activation of SHIP have now been reported and have been shown to exert an anti-inflammatory effect in vitro and in vivo (Harris et al. 2008). Moreover, these molecules have been successfully used to kill multiple myeloma cells in vitro indicating that SHIP agonists could be effective anticancer agents. Small molecule inhibitors of SHIP have also been reported which, consistent with observations from SHIP deleted mice, led to increased MDSCs and protected against GVHD. SHIP inhibitors increased levels of granulocytes, red blood cells, neutrophils, and platelets (Harris et al. 2008) in mice and could therefore have application to improve blood cell number in patients with myelodysplastic syndrome or myelosuppressive infection. A SHIP inhibitor also triggered the apoptosis of human acute myeloid leukemia cell lines, consistent with SHIP being antiapoptotic under some circumstances (Brooks et al. 2010)

Conclusion

SHIP is largely confined to hematopoietic cells and hydrolyzes the PI3K-generated second messenger $PI(3,4,5)P_3$, to $PI(3,4)P_2$. As a consequence, SHIP is able to modulate PI3K-/Akt-mediated signaling and hence the proliferation, differentiation, survival, activation, and migration of hematopoeitic cells. SHIP possesses a centrally located catalytic domain responsible for the hydrolysis of the 5′-phosphate of the membrane phosphoinositide lipid $PI(3,4,5)P_3$. Moreover, the presence of multiple structural domains that facilitate protein-protein interactions and cellular relocalization upon receptor stimulation allows SHIP to fulfill a scaffolding role independently of its catalytic function. The ability of PH domain-containing proteins to distinguish between different 3′-phosphoinositide lipids allows SHIP to act as a switch to redirect PI3K signaling toward $PI(3,4)P_2$-dependent effectors that are distinct from $PI(3,4,5)P_3$-dependent events. Thus, SHIP may function to fine-tune phosphoinositide signaling, rather than terminate it. SHIP can regulate signaling pathways elicited by engagement of appropriate receptors with cytokines, chemokines, antigens, and IgG in both lymphoid and myeloid cells. It plays a key role in the generation of lymphocyte and myeloid subsets and maintaining a balance between inflammatory and regulatory cells. Additionally, SHIP plays an important regulatory role in establishing endotoxin tolerance in macrophages, as well as regulating leukocyte polarization during migratory responses to

chemoattractants. The expression of SHIP is often lost, downregulated, or mutated in many cancer cells and is often targeted by pathogens to avoid detection and destruction by the immune system. Hence, modulation of SHIP offers potential for therapeutic intervention in an inflammatory/autoimmune and infectious disease as well as in cancer and transplantation settings.

Cross-References

- ▶ FcγRIIb
- ▶ Macrophages, Oxidative Stress, and Atherosclerosis
- ▶ Tregs in the Liver

References

Avota E, Harms H, Schneider-Schaulies S. Measles virus induces expression of SIP110, a constitutively membrane clustered lipid phosphatase, which inhibits T cell proliferation. Cell Microbiol. 2006;8:1826–39.

Brooks R, Fuhler GM, Iyer S, Smith MJ, Park MY, Paraiso KH, Engelman RW, Kerr WG. SHIP1 Inhibition increases immunoregulatory capacity and triggers apoptosis of hematopoietic cancer cells. J Immunol. 2010;184:3582–9.

Charlier E, Conde C, Zhang J, Deneubourg L, Di Valentin E, Rahmouni S, Chariot A, Agostinis P, Pang PC, Haslam SM, Dell A, Penninger J, Erneux C, Piette J, Gloire G. SHIP-1 inhibits CD95/APO-1/Fas-induced apoptosis in primary T lymphocytes and T leukemic cells by promoting CD95 glycosylation independently of its phosphatase activity. Leukemia. 2010;24:821–32.

Costinean S, Sandhu SK, Pedersen IM, Tili E, Trotta R, Perrotti D, Ciarlariello D, Neviani P, Harb J, Kauffman LR, Shidham A, Croce CM. Src homology 2 domain-containing inositol-5-phosphatase and CCAAT enhancer-binding protein beta are targeted by miR-155 in B cells of emicro-MiR-155 transgenic mice. Blood. 2009;114:1374–82.

Ghansah T, Paraiso KH, Highfill S, Desponts C, May S, McIntosh JK, Wang JW, Ninos J, Brayer J, Cheng F, Sotomayor E, Kerr WG. Expansion of myeloid suppressor cells in SHIP-deficient mice represses allogeneic T cell responses. J Immunol. 2004;173:7324–30.

Hamilton MJ, Ho VW, Kuroda E, Ruschmann J, Antignano F, Lam V, Krystal G. Role of SHIP in cancer. Exp Hematol. 2011;39:2–13.

Harris SJ, Parry RV, Westwick J, Ward SG. Phosphoinositide lipid phosphatases: natural regulators of phosphoinositide 3-kinase signaling in T lymphocytes. J Biol Chem. 2008;283:2465–9.

Helgason CD, Damen JE, Rosten P, Grewal R, Sorensen P, Chappel SM, Borowski A, Jirik F, Krystal G, Humphries RK. Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. Genes Dev. 1998;12:1610–20.

Kerr WG. A role for SHIP in stem cell biology and transplantation. Curr Stem Cell Res Ther. 2008;3:99–106.

Kerr WG. Inhibitor and activator: dual functions for SHIP in immunity and cancer. Ann N Y Acad Sci. 2011;1217:1–17.

Lemmon MA, Ferguson KM. Signal-dependent membrane targeting by pleckstrin homology (PH) domains. Biochem J. 2000;350(Pt 1):1–18.

Leung WH, Tarasenko T, Bolland S. Differential roles for the inositol phosphatase SHIP in the regulation of macrophages and lymphocytes. Immunol Res. 2009;43:243–51.

Lioubin MN, Myles GM, Carlberg K, Bowtell D, Rohrschneider LR. Shc, Grb2, Sos1, and a 150-kilodalton tyrosine-phosphorylated protein form complexes with Fms in hematopoietic cells. Mol Cell Biol. 1994;14:5682–91.

Liu L, Damen JE, Cutler RL, Krystal G. Multiple cytokines stimulate the binding of a common 145-kilodalton protein to Shc at the Grb2 recognition site of Shc. Mol Cell Biol. 1994;14:6926–35.

Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;129:1261–74.

Nguyen NY, Maxwell MJ, Ooms LM, Davies EM, Hilton AA, Collinge JE, Hilton DJ, Kile BT, Mitchell CA, Hibbs ML, Jane SM, Curtis DJ. An ENU-induced mouse mutant of SHIP1 reveals a critical role of the stem cell isoform for suppression of macrophage activation. Blood. 2011;117:5362–71.

Ono M, Bolland S, Tempst P, Ravetch JV. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. Nature. 1996;383:263–6.

Ooms LM, Horan KA, Rahman P, Seaton G, Gurung R, Kethesparan DS, Mitchell CA. The role of the inositol polyphosphate 5-phosphatases in cellular function and human disease. Biochem J. 2009;419:29–49.

Peng Q, Malhotra S, Torchia JA, Kerr WG, Coggeshall KM, Humphrey MB. TREM2- and DAP12-dependent activation of PI3K requires DAP10 and is inhibited by SHIP1. Sci Signal. 2010;3:ra38.

Shenker BJ, Dlakic M, Walker LP, Besack D, Jaffe E, LaBelle E, Boesze-Battaglia K. A novel mode of action for a microbial-derived immunotoxin: the cytolethal distending toxin subunit B exhibits phosphatidylinositol 3,4,5-triphosphate phosphatase activity. J Immunol. 2007;178:5099–108.

Sjögren's Syndrome

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Synonyms

Autoimmune epithelitis; Gougerot's syndrome; Sicca syndrome

Definition

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands (mainly the lacrimal and salivary glands), leading to dry eyes and dry mouth (also known as sicca symptoms), as a result of impaired secretions. The disease can also affect virtually all exocrine glands of the body, such as nose, throat, bronchi, trachea, skin, and vagina. Extraglandular manifestations and non-Hodgkin lymphoma may occur in about one third and in 5–8 % of SS patients respectively.

SS can occur alone (primary SS) or with other autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, systemic or limited sclerosis, and autoimmune thyroid disease. The term "SS associated with other disease" has been recently proposed to replace the term "secondary SS" which traditionally was implemented to describe sicca features following the diagnosis of other connective tissue diseases such as rheumatoid arthritis.

Epidemiology

Sjögren's syndrome has a prevalence of 0.5–1 % of the general population and affects predominantly middle-aged females (women to men ratio of 9–1).

Diagnosis

Subjective Evaluation

The diagnosis of SS is based on a combination of findings from a detailed medical and family history, alcohol and smoking habits, intake of medications and other substances, physical examination findings, and laboratory test results; occasionally, minor salivary gland biopsies are available to support the diagnosis. Specific questionnaires addressing the presence of symptoms of oral and ocular dryness can also be helpful (Mavragani and Moutsopoulos 2009).

Objective Tests for Oral and Ocular Dryness

Ocular component

- Schirmer's test: This test evaluates the function of the lacrimal glands by measuring the amount of tears produced. A thin filter paper strip is placed on the back of the lower lid and measures the wetting of the film after 5 min. In patients with SS, wetting of the filter strip does not exceed 5 mm in 5 min.
- Rose Bengal or Lisamine Green staining: These tests reveal the presence of epithelial damage as the result of tear deprivation (keratoconjunctivitis sicca). A drop of dye solution is placed in the lower conjunctiva; the pigmented lesions detected by slit lamp exam reflect the affected areas of the conjunctiva and cornea.
- Break-up time (BUT): This is a nonspecific test assessing the quality of the tears. Under physiological conditions, the tear film breaks at least after 10 s. In SS, this test is usually reduced to less than 10 s, an indication of tear instability.

Oral component

- Sialometry: Saliva secretion is measured in a graded tube; it is considered abnormal for volumes less than 1.5 mL/15 min.
- Salivary gland scintigraphy: Reduced technetium 99 m pertechnetate uptake reflects salivary gland dysfunction. Its use is limited in clinical practice, given the high cost and the nonspecific results.
- Parotid sialography: In patients with SS, this test discloses alteration of the normal

structural pattern of parotid ductules. It is painful, poorly specific, and therefore, it is rarely used.

- Ultrasonography/MRI sialography/MRI: These are promising imaging techniques which need further validation (Tzioufas and Moutsopoulos 2008).
- Labial salivary gland biopsy: This is a relatively simple diagnostic procedure aimed at removal of minor salivary glands. Lymphocytic infiltrates around salivary gland epithelium are the hallmark of SS. A focus score ≥ 1 (defined as the presence of at least 50 lymphocytes/4 mm^2) is included in the classification criteria for the disease.

Serological Evaluation

Serological testing for the presence of autoantibodies [rheumatoid factor (~60 %), ANA (~90 %), anti-Ro/SSA (~60 %) and/or anti-La/SSB (~50 %)], hypergammaglobulinemia, chronic viral infections (HCV, HIV, HTLV-1), and thyroid function provides helpful insights for the disease diagnosis. The role of antibodies to muscarinic receptors in the pathogenesis of sicca features through mechanisms of autonomic dysfunction is emerging.

Classification Criteria

The most widely accepted criteria for the classification of SS are the "European American consensus group criteria," which require symptoms and signs of mucosal dryness along with the presence of either a focus score of 1 or more in minor salivary glands or the presence of anti-Ro/SSA and/or anti-La/SSB autoantibodies (Table 1) (Peri et al. 2012).

Sjögren's Syndrome, Table 1 European American Consensus Group criteria

1. Ocular symptoms (at least one present)
Persistent, troublesome dry eyes every day for longer than 3 months
Recurrent sensation of sand or gravel in the eyes
Use of a tear substitute more than three times a day
2. Oral symptoms (at least one present)
Feeling of dry mouth every day for at least 3 months
Recurrent feeling of swollen salivary glands as an adult
Need to drink liquids to aid in swallowing dry food
3. Objective evidence of dry eyes (at least one present) (see how at pages 7 and 8)
Schirmer's test $\leq$5 mm/5 min
Van Bijsterveld score $\geq$4 (after lissamin test)
4. Objective evidence of salivary-gland involvement (at least one present)
Salivary-gland scintigraphy
Parotid sialography
Unstimulated salivary flow ($\leq$1.5 mL per 15 min, $\leq$0.1 mL/min)
5. Histological features
Positive minor salivary-gland biopsy sample (focus score $\geq$1 : This refers to a cluster of 50 or more lymphocytes per lobule when at least four lobules are assessed)
6. Autoantibodies
Presence of antibodies to Ro/SSA or to La/SS-B
Classification of primary Sjögren's syndrome requires four of six criteria, including a positive minor salivary-gland biopsy sample or antibody to Ro/SSA and/or to La/SSB, or three of the four objective criteria (criteria 3–6)
Classification of secondary Sjögren's syndrome requires an established connective-tissue disease and one sicca symptom (criteria 1 or 2) plus any 3 of the four objective criteria (Items 3, 4, 5)
Exclusions include previous radiotherapy to the head and neck, lymphoma, sarcoidosis, graft-versus-host disease, and infection with hepatitis C virus, or HIV, use of anticholinergic drugs

Differential Diagnosis

Diseases and conditions mimicking SS include, among others, medications that can cause sicca symptoms (e.g., antihistamines, nasal decongestants, diuretics, antidiarrhoeals, antipsychotics, sedatives, antidepressants), sarcoidosis, infections (HCV, HIV), hyperlipoproteinemias (types II, IV, and V), diabetes mellitus, irradiation and the IgG4-related sialadenitis (formerly known as Mikulicz disease or as Kuttner's tumor) (Ramos-Casals et al. 2007).

Clinical Manifestations

The clinical features of SS are divided into those related to exocrine dysfunction (glandular) and to

those affecting other organs beyond exocrine glands (extraglandular or systemic). The latter can be further divided into those characterized by periepithelial infiltrates in parenchymal organs (kidney, lung, liver) and to those derived by immunocomplexes deposition as a result of B cell hyperactivity (glomerulonephritis, purpura, peripheral neuropathy) (Mavragani and Moutsopoulos 2010; Skopouli et al. 2000).

Glandular Features

- Dry eyes: Symptoms include burning, itching, or sand feeling in the eyes, which may be red and irritated. The vision may be blurry.
- Dry mouth: Difficulty in chewing, swallowing and speaking, adherence of food to the buccal surfaces, and abnormalities of taste and smell are common manifestations. Moreover, lack of saliva protection increases the chances of fungal stomatitis and dental caries. The tongue appears dry and fissured.
- Salivary gland swelling: This is a frequent SS-related symptom, associated with increased risk for the development of lymphoma. Differential diagnosis includes superimposed infection, metabolic derangement, infiltrative disorders, and IgG4-related sialadenitis.
- Dry cough/xerotrachea: These are mainly related to the desiccation of tracheobronchial tree. Pulmonary function tests usually reveal a small airway obstructive pattern which is thought to result from peribronchial and/or peribronchiolar lymphocytic infiltrates. A "dirty lung" appearance on a chest-X-ray and peribronchial thickening on high-resolution computed tomography imaging are the main imaging findings observed.
- Vaginal dryness
- Dry skin

Extraglandular Features

Musculoskeletal

Arthralgias, non-erosive arthritis, and myalgias are common in SS. Fibromyalgia and chronic fatigue are also common, affecting the quality of life of these patients.

Raynaud's Phenomenon

Raynaud's phenomenon, which may occur prior to the appearance of sicca symptoms, affects about one third of SS patients.

Respiratory Tract Involvement

The majority of pulmonary symptoms are related to glandular involvement of the trachea and bronchial glands. Less frequently interstitial lung disease is diagnosed.

Gastrointestinal and Hepatobiliary Manifestations

Dysphagia related to esophageal dysmotility and reduced saliva is observed in SS patients. Hoarseness as a result of gastroesophageal and laryngopharyngeal reflux is also observed. Liver involvement mainly in the form of primary biliary cirrhosis (PBC) has been described in around 5–10 % of SS patients often associated with the presence of serum anti-mitochondrial (AMA) antibodies.

Renal Involvement

Two types of renal involvement have been described and include tubulointerstitial nephritis and glomerulonephritis. The former is more common and mild, expressed as hypokalemia, low urine specific gravity (hyposthenuria), and alkaline urine pH. Glomerulonephritis is rather rare, usually occurring in association with systemic vasculitis, hypocomplementemia, and cryoglobulinemia; these features increase the risk for the development of lymphoma. IgM and complement are detected in renal biopsy by immunofluorescence.

Vasculitis

Vasculitis affecting small vessels of the skin occurs in about 15 % of SS patients, usually many years after the diagnosis of SS. Palpable purpura and more rarely urticarial lesions are the most common clinical manifestations, often in association with cryoglobulins, high titers of rheumatoid factor, antinuclear antibodies (ANA), hypocomplementemia, and hypergammaglobulinemia. Less frequently, peripheral nerves, muscles, and kidneys are affected. Vasculitic lesions in the context of SS are

associated with increased risk for the development of lymphoma and increased mortality.

Neuropsychiatric Involvement

The prevalence of peripheral neuropathy in SS displays a wide variability between studies; the main subtypes observed are purely sensory (stocking-glove distribution), small fiber neuropathy (SFN), expressed as painful and burning paresthesias, and sensorimotor neuropathy (Pavlakis et al. 2012).

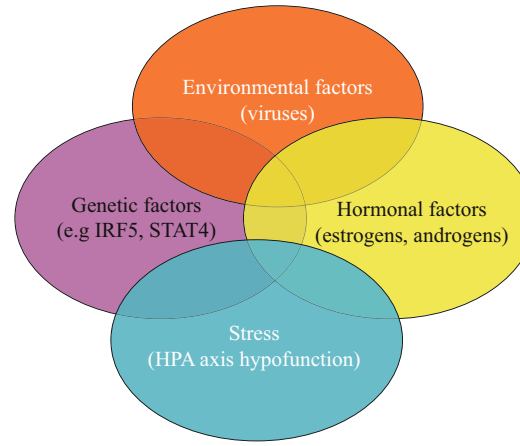
Central nervous system involvement in primary SS remains controversial. While earlier studies reported high prevalence of CNS pathology in SS (including demyelinating lesions that may mimic multiple sclerosis), subsequent reports failed to confirm these findings. Finally, compared to healthy individuals, SS patients reportedly display psychopathological abnormalities and distinct personality traits such as neuroticism, psychoticism, and obsessiveness (Mavragani and Moutsopoulos 2010).

Lymphoma

Non-Hodgkin B cell lymphoma occurs in approximately 5–8 % of patients with SS, conferring increased mortality. Mucosa-associated lymphoid tissue (MALT) type is the most common type; however, follicular lymphoma, lymphoplasmacytoid, and diffuse large B cell lymphoma (DLBCL) have also been described. Adverse prognostic factors associated with the development of lymphoma include lymphopenia, low C4 serum levels, cryoglobulinemia, peripheral neuropathy, glomerulonephritis, vasculitis, salivary gland enlargement, and the presence of germinal centers at salivary gland biopsy. Lymphoma complicating SS most commonly involve the salivary glands. Other organs, however, such as stomach, lung, nasopharynx, and thyroid, can also be involved. In high-risk patients, close follow-up for the development of lymphoma is appropriate (Voulgarelis et al. 2012; Ioannidis et al. 2002).

Etiopathogenesis

As proposed for a number of other autoimmune diseases, SS probably results from a complex interplay between genetic, environmental, hormonal, and stress factors, leading to aberrant epithelial cell activation (Fig. 1).



Sjögren's Syndrome, Fig. 1 The underlying mechanism of Sjogren's syndrome pathogenesis is a complex interplay between genetic, hormonal, environmental, and psychopathological factors. *HPA* hypothalamic-pituitary axis

Genetics

Genetic predisposition of SS is supported by the observed familial clustering and the increased frequency of certain alleles implicated in innate and adaptive responses inside and outside the HLA locus. The presence of polymorphisms in the MHC II, the interferon regulating factor-5 (IRF5), and signal transducer and activator of transcription 4 (STAT4) genes have been associated with disease susceptibility (Ice et al. 2012).

Stress

The occurrence of stressful events prior to disease onset and defective coping strategies in conjunction with the presence of a hypoactive hypothalamic-pituitary-adrenal axis imply stress as a potential trigger in disease pathogenesis.

Hormonal Factors

Disease onset around menopause and the female predominance support the contributory role of hormones in disease pathogenesis. SS patients are characterized by low androgen and estrogen levels which have been previously shown to

protect salivary epithelium apoptosis; development of SS-like disease in mice lacking estrogens is also supportive (Konttinen et al. 2012).

Viruses

Activation of the type I interferon pathway in SS, as confirmed by gene expression, serological, and immunohistochemical studies, strongly suggests a role for viral infection in disease pathogenesis. Thus far, several viruses and viral particles have been implicated including Coxsackie virus, EBV, HTLV-1, HCV, CMV, HHV-6, and HHV-8, among others (Sipsas et al. 2011).

Pathophysiology

According to the currently proposed pathophysiological scenario for SS, epitheliotropic viruses or endogenous nucleic acids might serve as initial triggers of epithelial activation, a central event in disease pathogenesis (autoimmune epithelitis). The latter is evidenced by increased expression of several immunomodulatory molecules implicated in:

- Lymphocyte recruitment/homing/expansion/differentiation (proinflammatory and immunoregulatory cytokines, lymphoid chemokines, BAFF, adhesion molecules)
- Apoptosis (FaS, FaSL, Perforin, granzymes)
- Ag-presentation (MHC molecules, B7 costimulatory molecule, translocation of nuclear autoantigens)
- Sensing innate stimuli (TLRs, CD91)

As a result of increased apoptosis, exposure of autoantigens, such as Ro/SSA and La/SSB, and subsequent production of disease-specific autoantibodies with immunocomplexes formation can lead to robust type I interferon (IFN) production by plasmacytoid dendritic cells (PDCs), the "professional IFNα producers" in genetically susceptible individuals. Type I IFN along with low estrogen/androgen status might further activate epithelial cells, leading to BAFF overexpression and autoantibody production. The activated epithelial cell acts as an antigen-presenting cell and through upregulation of chemotactic molecules may lead to further recruitment of inflammatory cells, activation of T and B cells, and autoantibody production. Tissue damage, occasionally associated with B cell monoclonal expansion, may follow (Jonsson et al. 2011; Manoussakis and Kapsogeorgou 2010; Mariette and Gottenberg 2010; Nikolov and Illei 2009; Tzioufas et al. 2012; Vakaloglou and Mavragani 2011) (Fig. 2).

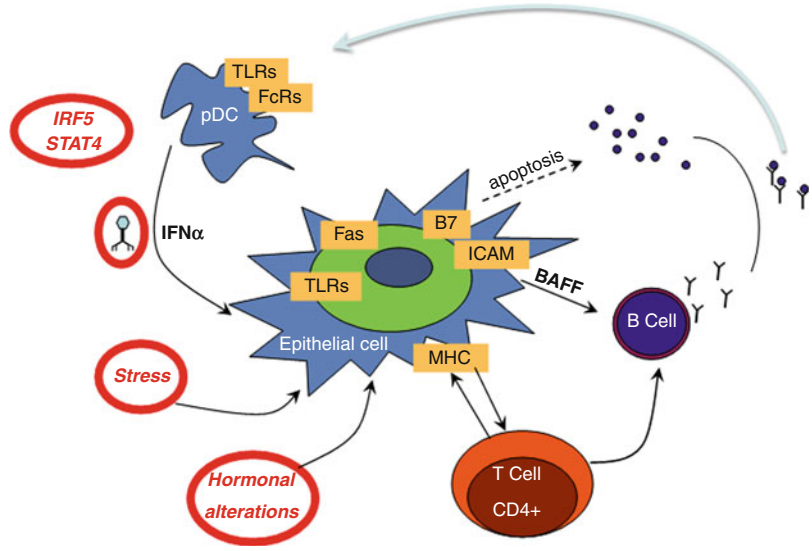
Therapy

Treatment of SS is mainly symptomatic, aimed at relief of mucosal dryness. Avoiding dry environments and dehydration along with artificial tears, lubricative ointments, ocular cyclosporine drops, and occlusion of the punctae lacrimales are the main modalities implemented in the management of keratoconjunctivitis sicca. Muscarinic agonists, including pilocarpine and cevimeline hydrochloride, have been proved efficacious, increasing saliva and tear secretion.

Immunomodulatory agents are generally reserved for the treatment of extraglandular SS manifestations. Hydroxychloroquine may be effective for arthralgias, arthritis, and myalgias, while corticosteroids, methotrexate, azathioprine, and cyclophosphamide have been tried for persistent arthritis, interstitial lung disease, peripheral nerve involvement, severe vasculitis, and renal involvement. Cytotoxic drugs, however, should be administered with extreme caution since they may increase the risk of lymphoma, particularly in the high-risk groups.

Given the contributory role of B cells in disease pathogenesis, B cell depletion strategies provide a rational therapeutic approach. Anti-CD20 administration seems promising in the management of several extraglandular disease manifestations, including lymphoma. Such agents may also reduce fatigue and mucosal dryness in the presence of residual glandular function.

Studies suggest that anti-TNF agents (e.g., infliximab and etanercept) are not effective in SS; augmentation of the already activated type I interferon-BAFF pathway is a plausible explanation (Ramos-Casals et al. 2012; Ramos-Casals et al. 2010).



Sjögren's Syndrome, Fig. 2 The pathophysiology of Sjogren's syndrome. *FcRs* Fc Receptors, *ICAM* intracellular adhesion molecule, *TLR* toll-like receptors

Cross-References

- Complement in Rheumatic Diseases
- Micro-RNA in Autoimmunity
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Ice JA, Li H, Adrianto I, Lin PC, Kelly JA, Montgomery CG, Lessard CJ, Moser KL. Genetics of Sjogren's syndrome in the genome-wide association era. J Autoimmun. 2012;39:57–63.

Ioannidis JP, Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjogren's syndrome. Arthritis Rheum. 2002;46:741–7.

Jonsson R, Vogelsang P, Volchenkov R, Espinosa A, Wahren-Herlenius M, Appel S. The complexity of Sjogren's syndrome: novel aspects on pathogenesis. Immunol Lett. 2011;141:1–9.

Konttinen YT, Fuellen G, Bing Y, Porola P, Stegaev V, Trokovic N, Falk SS, Liu Y, Szodoray P, Takakubo Y. Sex steroids in Sjogren's syndrome. J Autoimmun. 2012;39:49–56.

Manoussakis MN, Kapsogeorgou EK. The role of intrinsic epithelial activation in the pathogenesis of Sjogren's syndrome. J Autoimmun. 2010;35:219–24.

Mariette X, Gottenberg JE. Pathogenesis of Sjogren's syndrome and therapeutic consequences. Curr Opin Rheumatol. 2010;22:471–7.

Mavragani CP, Moutsopoulos HM. The geoepidemiology of Sjogren's syndrome. Autoimmun Rev. 2009;9: A305–10.

Mavragani CP, Moutsopoulos HM. The geoepidemiology of Sjogren's syndrome. Autoimmun Rev. 2010;9: A305–10.

Nikolov NP, Illei GG. Pathogenesis of Sjogren's syndrome. Curr Opin Rheumatol. 2009;21:465–70.

Pavlakis PP, Alexopoulos H, Kosmidis ML, Mamali I, Moutsopoulos HM, Tzioufas AG, Dalakas MC. Peripheral neuropathies in Sjogren's syndrome: a critical update on clinical features and pathogenetic mechanisms. J Autoimmun. 2012;39:27–33.

Peri Y, Agmon-Levin N, Theodor E, Shoenfeld Y. Sjogren's syndrome, the old and the new. Best Pract Res Clin Rheumatol. 2012;26:105–17.

Ramos-Casals M, Brito-Zeron P, Font J. Lessons from diseases mimicking Sjogren's syndrome. Clin Rev Allergy Immunol. 2007;32:275–83.

Ramos-Casals M, Tzioufas AG, Stone JH, Siso A, Bosch X. Treatment of primary Sjogren syndrome: a systematic review. JAMA. 2010;304:452–60.

Ramos-Casals M, Brito-Zeron P, Siso-Almirall A, Bosch X, Tzioufas AG. Topical and systemic medications for the treatment of primary Sjogren's syndrome. Nat Rev Rheumatol. 2012;8:399–411.

Sipsas NV, Gamaletsou MN, Moutsopoulos HM. Is Sjogren's syndrome a retroviral disease? Arthritis Res Ther. 2011;13:212.

Skopouli FN, Dafni U, Ioannidis JP, Moutsopoulos HM. Clinical evolution, and morbidity and mortality of primary Sjogren's syndrome. Semin Arthritis Rheum. 2000;29:296–304.

Tzioufas AG, Moutsopoulos HM. Ultrasonography of salivary glands: an evolving approach for the diagnosis of Sjogren's syndrome. Nat Clin Pract Rheumatol. 2008;4:454–5.

Tzioufas AG, Kapsogeorgou EK, Moutsopoulos HM. Pathogenesis of Sjogren's syndrome: what we know and what we should learn. J Autoimmun. 2012.

Vakaloglou KM, Mavragani CP. Activation of the type I interferon pathway in primary Sjogren's syndrome: an update. Curr Opin Rheumatol. 2011;23:459–64.
Voulgarelis M, Ziakas PD, Papageorgiou A, Baimpa E, Tzioufas AG, Moutsopoulos HM. Prognosis and outcome of non-Hodgkin lymphoma in primary Sjogren syndrome. Medicine (Baltimore). 2012;91:1–9.

Skin in Systemic Lupus Erythematosus

Pathogenesis, Clinical Manifestations, and Treatment

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Synonyms

ACR criteria; Cutaneous lupus erythematosus; Photosensitivity; RCLASI; Skin; SLICC index; Treatment

Definition

The autoimmune disease lupus erythematosus (LE) shows many different clinical manifestations reaching from primarily cutaneous lesions (cutaneous lupus erythematosus, CLE) to severe systemic organ manifestations (systemic lupus erythematosus, SLE). Skin manifestations appear in 73–85 % of patients with SLE and may occur at any stage of the disease; conversely, only a small percentage of patients with CLE subsequently develop a systemic manifestation of the disease.

Introduction

In 1982, the American College of Rheumatology (ACR) introduced a set of criteria for the classification of SLE that provided some degree of uniformity in classifying the patient populations of clinical studies (Tan et al. 1982). Although 4 of the 11 ACR criteria are mucocutaneous manifestations (malar rash, discoid lesions, photosensitivity, and oral ulcers), the clinical presentation of skin involvement in SLE presents with a much broader spectrum. Importantly, photosensitivity is not specific for SLE, and it is observed in other conditions, such as polymorphous light eruption (PLE) (Albrecht et al. 2004). In 2012, the Systemic Lupus Collaborating Clinics (SLICC) revised and validated the ACR criteria in order to improve clinical relevance and incorporate new knowledge in SLE immunology (Petri et al. 2012). Moreover, the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) was revised in 2010, taking into account both anatomical regions (e.g., face, chest, arms) and morphological aspects (e.g., erythema, edema/infiltration, scarring/atrophy) to evaluate "activity" and "damage" of skin manifestations in all subtypes of CLE (Kuhn et al. 2010b). Although several agents are approved for the treatment of SLE, including the novel monoclonal antibody belimumab (Manzi et al. 2012), a B lymphocyte stimulator-specific inhibitor, no drugs have been licensed specifically for the treatment of skin manifestations of the disease. Thus, topical and systemic agents in CLE are mostly used "off-label." In this entry, recent information on pathogenesis, clinical manifestations, and treatment of CLE are summarized.

Pathogenesis

Cutaneous manifestations of autoimmune diseases may be caused by autoantibodies and immune complexes, independent of the question

Comment: As the number of references is limited in the "Encyclopedia of Medical Immunology," mainly review articles are cited. If the original articles are of interest, please consult the original articles referred to in the review articles.

of whether skin lesions are the primary sign of the disease, as in CLE, or part of a disease spectrum, as in SLE (Liu and Davidson 2012). In fact, immune complex deposition and complement activation can be demonstrated in tissue samples of both CLE and SLE patients (Kuhn et al. 2010a). As skin lesions mainly occur in sun-exposed areas, such as the face, upper back, V area of the neck, and extensor aspects of the arms, it is commonly accepted that sun exposure can induce and exacerbate cutaneous manifestations in patients with all subtypes of CLE and SLE, which supports the role of ultraviolet (UV) light in the pathogenesis of the disease (Kuhn et al. 2010d). The mechanisms by which UV irradiation activates the autoimmune response are not completely understood. However, recent studies assessing the molecular mechanisms, which underlie UV-induced apoptosis, contribute to a better understanding of the disease pathogenesis (Lehmann and Homey 2009). In primary and UV-induced skin lesions of patients with CLE, compared with healthy controls, a significant increase of apoptotic keratinocytes has been found by immunohistochemistry (Baima and Sticherling 2001; Kuhn et al. 2010a). In the majority of patients with CLE, the number of apoptotic nuclei in the epidermis already increased significantly between day 1 and day 3 after a single UV exposure (Kuhn et al. 2006). These data suggest that accumulation of apoptotic cells or a deficiency in clearance might play an important role in the development of skin lesions. It has further been demonstrated that the cytoplasmatic molecule, annexin 1, which is externalized during apoptosis to the outer leaflet of the plasma membrane, leads to the suppression of dendritic cells (Weyd et al. 2013; Arur et al. 2003). A recent study showed that the percentage of anti-annexin 1 antibodies was significantly higher in the sera of patients with CLE compared to healthy controls (Kretz et al. 2010); however, the functional role of anti-annexin 1 antibodies in this disease is not known. The results of this study may suggest that blocking anti-annexin 1 antibodies might interfere with the generation of tolerance and support the development of autoimmunity.

Clinical Manifestations

The clinical expression of the skin lesions in SLE shows a great variety and has led to the practice of identifying different subtypes of the disease. However, the development of a unified classification of skin manifestations has proven difficult. The classification system developed by Gilliam in 1977 divided cutaneous lesions into LE-specific and LE-nonspecific manifestations by histological analysis of skin biopsy specimens (Gilliam 1977). Skin lesions such as urticarial vasculitis and livedo reticularis are some of the most common LE-nonspecific cutaneous lesions and are primarily associated with active SLE, reflecting potentially serious complications (Costner et al. 2003). In contrast, the LE-specific cutaneous findings encompass the various subtypes of CLE, which were subdivided into three different categories defined by constellations of clinical features, histological changes, serological abnormalities, and average duration of skin lesions: acute cutaneous LE (ACLE), subacute CLE (SCLE), and chronic cutaneous LE (CCLE), including discoid LE (DLE), chilblain LE (CHLE), and LE panniculitis (LEP). After the initial description of Gilliam's nomenclature more than three decades ago, this classification system has been modified in 2004 (Kuhn and Ruzicka 2004). An additional subtype with characteristic clinical, histological, and photobiological features, termed LE tumidus (LET), was first described in 1909 and has only recently been defined as a separate entity of CLE (Table 1).

Acute Cutaneous Lupus Erythematosus (ACLE)

ACLE may occur as a localized, occasionally transient form or as a generalized widespread form of the disease (Costner et al. 2003). The more common localized form is characterized by the classical "butterfly rash" which is often misdiagnosed as sunburn. It is defined as a sharply bordered erythema with symmetrical distribution in the central part of the face, typically sparing the nasolabial folds. The less common generalized form of ACLE (5–10 %) is often associated with the occurrence of

Skin in Systemic Lupus Erythematosus, Table 1 Classification of cutaneous lupus erythematosus[a]

Acute cutaneous lupus erythematosus (ACLE)
Localized form
Generalized form
Subacute cutaneous lupus erythematosus (SCLE)
Annular form
Papulosquamous form
Chronic cutaneous lupus erythematosus (CCLE)
Discoid lupus erythematosus (DLE)
Localized form
Disseminated form
Lupus erythematosus profundus panniculitis (LEP)
Chilblain lupus erythematosus (CHLE)
Intermittent cutaneous lupus erythematosus (ICLE)
Lupus erythematosus tumidus (LET)

[a]Modified after (Kuhn and Ruzicka 2004)

systemic manifestations. Symmetrically distributed maculopapular, erythematous to violaceous, and sometimes pruritic lesions may involve the trunk, in particular the UV-exposed areas, but may also be localized on the hands or feet, while knuckles are typically spared. Telangiectases and periungual erythema can occur at the nail fold and are often associated with a red lunula. Usually, the generalized form of ACLE presents with increased disease activity of SLE, which may be accompanied by mucosal changes affecting the mouth (hard palate, buccal mucosa, gingiva, uvula), nose, pharynx, and vagina. In most of the cases, ACLE does not lead to depigmentation and is a non-scarring subtype, but diffuse thinning of the hair ("lupus hair") can occur along the hairline (Kuhn et al. 2007). The highly acute form of generalized ACLE characterized by toxic epidermal necrolysis (TEN)-like lesions is rarely seen in SLE patients.

Subacute Cutaneous Lupus Erythematosus (SCLE)

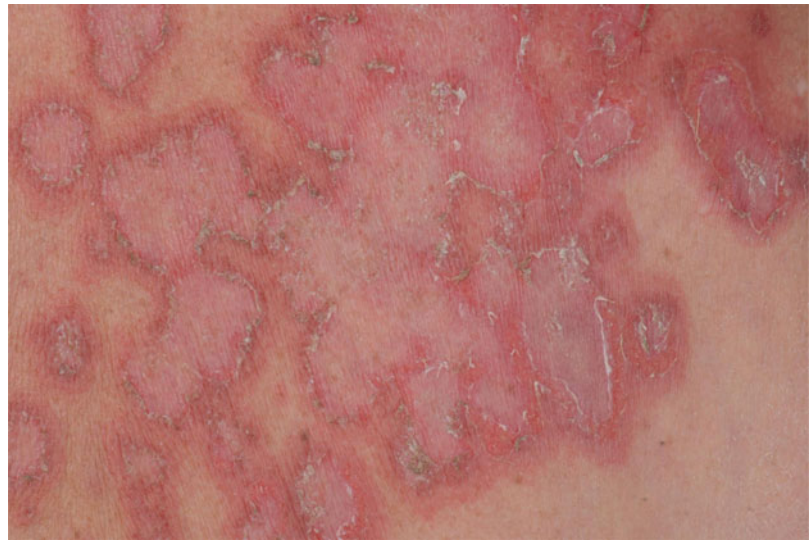
SCLE presents as erythematous macules or papules that expand and merge into hyperkeratotic papulosquamous or annular, sometimes polycyclic plaques with a garland-like appearance (Fig. 1). Two forms of this subtype can be distinguished: a papulosquamous variant consisting of psoriasis-like or eczema-like lesions and an annular variant consisting of slightly raised erythema with central clearing (Sontheimer 2005). Both forms may be found in the same patient. Typically, SCLE lesions are located on sun-exposed skin including the lateral aspects of the face, the V area of the neck (often with sparing of area under the chin), the upper ventral and dorsal part of the trunk, and the dorsolateral aspects of the arms. Skin lesions of SCLE can heal with vitiligo-like depigmentation, as a diagnostic "clue" of the disease (Kuhn et al. 2007). Similar to ACLE, a TEN-like picture can develop in some patients, especially after UV exposure. SCLE shows recurrent episodes in the majority of patients, and in 10–15 % it develops into a moderate form of SLE. In contrast to other subtypes of CLE, SCLE is often induced by drugs, for example, including hydrochlorothiazide, ACE inhibitors, and terbinafine. In drug-induced SCLE, skin lesions can be more widespread with extension to the lower extremities.

Chronic Cutaneous Lupus Erythematosus (CCLE)

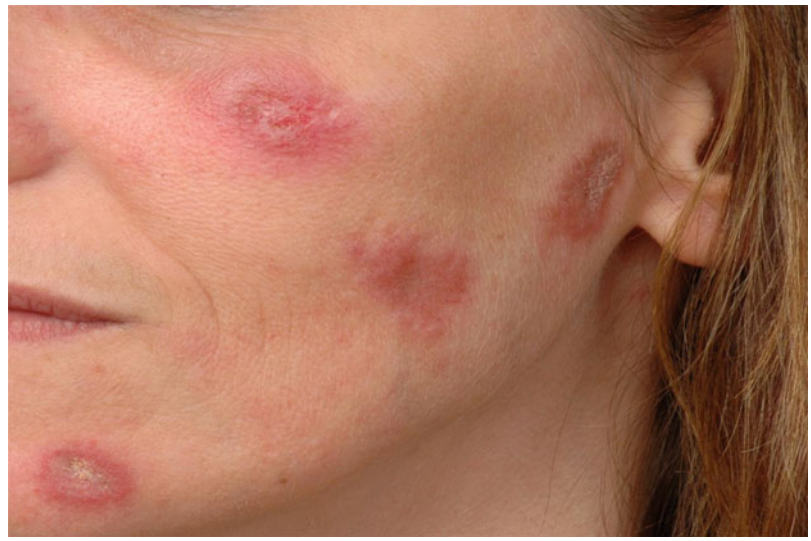
CCLE includes three different forms of the disease: discoid LE (DLE), LE profundus/panniculitis (LEP), and chilblain LE (CHLE).

Discoid Lupus Erythematosus (DLE)

DLE is the most common form of CCLE and consists of sharply bordered, coin-shaped ("discoid") erythematous plaques, on which adherent scales are present (Fig. 2). Removal of the scale shows follicle-sized keratotic spikes, the "carpet tack sign" (Kuhn et al. 2007). This form of CCLE can lead to atrophy and scarring with central depigmentation and peripheral hyperpigmentation. Periorally, characteristic pitting scars can develop. As hair follicles are irreversibly damaged, areas such as the scalp, eyebrows, and hairy regions of the face develop scarring alopecia. The localized form of DLE occurs in 80 % and has a predilection for the face and scalp, especially the cheeks, forehead, ears, nose, and upper lip (Costner et al. 2003). The disseminated form of DLE, involving mostly the



Skin in Systemic Lupus Erythematosus, Fig. 1 Subacute cutaneous lupus erythematosus (*SCLE*). Annular erythematous plaques with polycyclic confluence and central hypopigmentation on the upper back

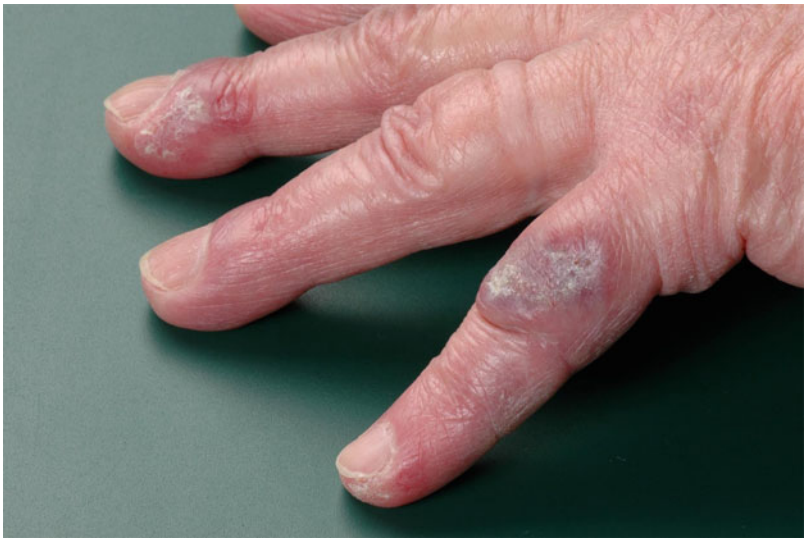


Skin in Systemic Lupus Erythematosus, Fig. 2 Discoid lupus erythematosus (*DLE*). Coin-shaped plaques with peripheral erythematous, hyperkeratotic border, and central atrophic scarring on the left cheek

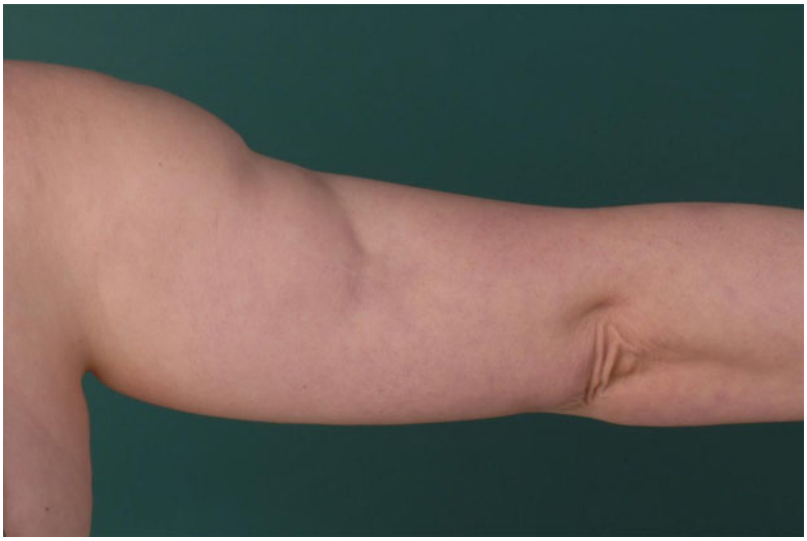
upper part of the trunk and the extensor surfaces of the extremities, is less common. Exposure to the sun or irritating stimuli (Koebner phenomenon) can provoke or exacerbate the disease (Ueki 2005).

Lupus Erythematosus Profundus/Panniculitis (LEP)

LEP is clinically characterized by painful subcutaneous nodules and plaques, which may later adhere to the overlying skin. At a later stage of the disease, the lesions can develop into asymptomatic lipatrophy (Fig. 3). Skin lesions of LEP are typically located in the gluteal region and on the thighs as well as on the upper extremities. The face, scalp, and chest may also be affected; the salivary glands are rarely involved. Periorbital edema may be an initial presenting symptom prior to the development of typical skin changes (Kuhn et al. 2007). In the course of disease, the nodules can develop ulcerations and deeply indented scars, which can be a major cosmetic concern to patients. In older lesions, calcification may occur. LEP can be induced by



Skin in Systemic Lupus Erythematosus, Fig. 3 Chilblain lupus erythematosus (*CHLE*). Erythematous plaques with livid infiltration or scaling on the fingers III–V of the left hand

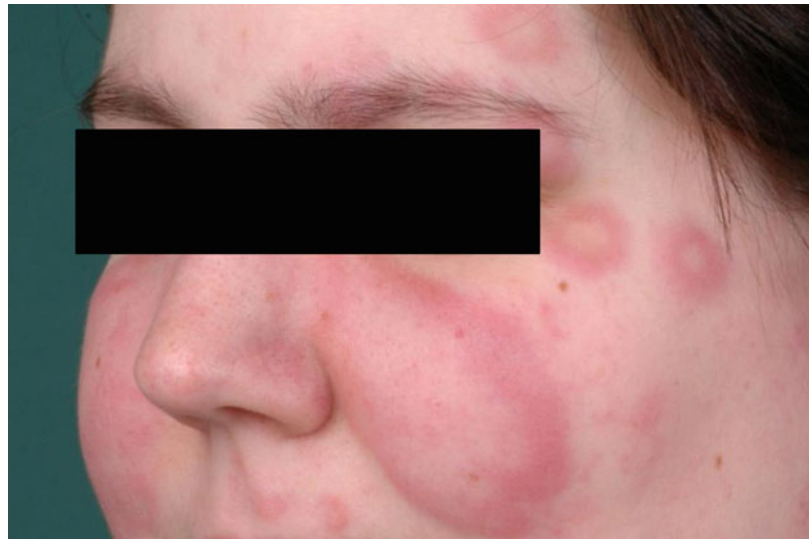


Skin in Systemic Lupus Erythematosus, Fig. 4 Lupus erythematosus panniculitis (*LEP*). Lipoatrophy on the right upper arm

irritative stimuli and is associated with DLE lesions in about 70 % of patients (Costner et al. 2003). The association with SLE is common and four or more of the ACR criteria are formally met in 35–50 % patients with LEP.

Chilblain Lupus Erythematosus (CHLE)

CHLE typically presents with tender, bluish plaques and nodules in cold-exposed areas (Fig. 4) (Kuhn et al. 2007). Edematous plaques and nodules may have central erosions or ulcerations, which affect the acral surfaces, especially the fingers, toes, heels, nose, and ears. In particular, CHLE appears during cold and damp times of the year or after a critical drop in temperature. Therefore, this subtype is clinically and histologically often difficult to distinguish from pernio ("chilblains"). The recent description of the genetic basis of a rare familial form of chilblain lupus has provided novel insights into the molecular pathogenesis and on understanding of the disease (Lee-Kirsch et al. 2006).



Skin in Systemic Lupus Erythematosus, Fig. 5 Lupus erythematosus tumidus (*LET*). Erythematous, edematous, urticaria-like plaques without epidermal involvement on the left facial area

Intermittent Cutaneous Lupus Erythematosus (ICLE)

Lupus Erythematosus Tumidus (LET)

On the basis of new clinical and scientific findings which identified specific clinical, histological, and photobiological criteria, LET was distinguished from CCLE in 2004 (Kuhn and Ruzicka 2004). Due to its benign, intermittent course, this subtype was included in the classification of CCLE as a separate entity, named ICLE. Clinically, LET is characterized by sharply bordered, "succulent," urticaria-like, erythematous papules and plaques with a smooth surface and without epidermal involvement (Kuhn et al. 2000) (Fig. 5). Skin lesions of this subtype are typically found on UV-exposed areas (face, upper back, chest, extensor surfaces of the upper arms) and may be annular, centrifugal, or crescent shaped and can, in rare cases, spread along the Blaschko's lines (Hinz et al. 2012). LET lesions may persist for months or resolve spontaneously without residual defects, such as scarring or dyspigmentation. Photosensitivity is a characteristic sign of this subtype, and skin lesions can be induced by UV light in more than 70 % of patients (Kuhn et al. 2001). Up to date, only isolated cases of LET associated with SLE have been reported. Therefore, LET has a good prognosis with a variable course of the disease in the majority of patients (Schmitt et al. 2010).

Bullous Lupus Erythematosus (BLE)

BLE is a rare subepidermal bullous disorder which is usually associated with acute and severe forms of SLE (Vassileva 2004). The skin lesions may present as solitary small vesicles or groups of vesicles or as large tense blisters on erythematous or normal skin. During the course of the disease, hyperpigmentation, milia, and scarring may occur. Clinically, BLE should be distinguished from bullous lesions arising from preexisting skin lesions in ACLE or at the margins of SCLE lesions. In addition, vesiculobullous skin changes have also been described as "TEN-like" ACLE and "TEN-like" SCLE, which are clinically similar to classic TEN. However, these diseases are usually not drug-induced but may rather be triggered, for example, by UV exposure. In a recent retrospective analysis from the international literature, it was highlighted that CLE with erythema multiforme (EM)- like or TEN-like lesions might be variants of already known CLE subpatterns (Torchia et al. 2012). The name "acute syndrome of apoptotic pan-epidermolysis (ASAP)" has also been suggested to define

a life-threatening acute cleavage of the epidermis resulting from hyperacute apoptotic injury of the epidermis (Ting et al. 2004).

Treatment of CLE

Prevention and Sunscreens

In all patients with SLE and CLE, it is necessary to provide instructions concerning methods of protection from sunlight and artificial sources of UV radiation and avoidance of potentially photosensitizing drugs (Kuhn et al. 2010d). As clinical evidence has proven a clear relationship between sun exposure and skin manifestations of SLE and CLE, experimental photoprovocation with different wavelengths has confirmed that cutaneous lesions can be induced by UV exposure (Kuhn et al. 2001). Therefore, patients should be advised that sunbathing and visiting tanning salons can induce the disease and exacerbate preexisting skin lesions. In SLE patients, even induction of systemic organ involvement, such as lupus nephritis, has been reported as a result of sun exposure (Kuhn et al. 2010d). It is recommended that UV irradiation on midday during summer should be avoided, as should traveling to sunny regions, and outdoor jobs with intensive sun exposure (e.g., roofer or gardener) should not be recommended to CLE patients. In addition, it needs to be considered that window glass is UVA permeable; therefore, sun exposure can induce skin lesions during a long car drive, depending on the tinge/coating of the glass and the duration of irradiation (Hampton et al. 2004). Consistent UV protection may also involve photoresistant clothing and thorough application of lightshielding substances with highly potent chemical (organic) and/or physical (inorganic) UVA- and UVB-protective filters (Hexsel et al. 2008). These substances should be used in sufficient amounts (approximately 2 mg/cm^2) and should contain a high sun protection factor (SPF 50). To avoid induction and exacerbation of cutaneous manifestations, sunscreens should be applied at least 20–30 min before sun exposure. In a recent randomized, double-blind, vehicle-controlled study, it was demonstrated that a broad-spectrum liposomal sunscreen with a very high sun protection factor can prevent damage induced by UV irradiation in photosensitive patients with CLE under standardized conditions (Kuhn et al. 2011a). Moreover, consistent sunscreen protection in patients with SLE is associated with significant better clinical outcomes, such as less frequent renal involvement and a decreased need for immunosuppressive treatment (Vila et al. 1999).

Topical Treatment

Topical Corticosteroids

Topical corticosteroids are the first-line treatment for skin manifestations in CLE and SLE. One randomized, controlled, 12-week crossover study exists according to the Cochrane Database of Systematic Reviews, which compares 0.05 % fluocinonide (a potent corticosteroid cream) with 1 % hydrocortisone (a low-potency corticosteroid cream) in 78 patients with DLE (Roenigk et al. 1980; Jessop et al. 2009). This trial demonstrated that high-potency corticosteroid cream is more effective than low-potency corticosteroid cream; the choice of the corticosteroid class depends on the location and the activity of the skin lesion (Kuhn et al. 2010c). Corticosteroid solution, lotion, or foam can be used in hairy areas, such as the scalp. Occlusive techniques (e.g., plastic food wrap, adhesive gas-permeable surgical dressings) and salicylic acid increase penetration and efficacy of topical corticosteroids. In skin lesions of localized DLE, intralesional injection of corticosteroids (triamcinolone acetonide 5–10 mg/ml) can be applied with high efficacy; however, there is a risk of subcutaneous atrophy (Kuhn et al. 2011c). Due to the well-known side effects, such as atrophy, telangiectasia, and steroid-induced rosacea-like dermatitis, treatment with topical corticosteroids should be time limited and intermittent.

Calcineurin Inhibitors

Calcineurin inhibitors, such as tacrolimus ointment and pimecrolimus cream, have shown efficacy in treating skin lesions of SLE and CLE

(Kuhn et al. 2010c). In a randomized, double-blind, bilateral comparison study, 0.1 % tacrolimus ointment showed a similar efficacy as 0.05 % clobetasol propionate in 18 patients with CLE lesions on the face (Tzung et al. 2007). Once weekly, microdermabrasion was added to potentiate the delivery of tacrolimus. In contrast to clobetasol propionate, tacrolimus ointment showed no side effects, such as telangiectasia. A further retrospective study compared the efficacy of a specially formulated preparation of tacrolimus 0.3 % in clobetasol propionate 0.05 % ointment in 13 therapy-resistant CLE patients with the application of only 0.1 % tacrolimus ointment in five patients (Madan et al. 2010). The combined preparation was more effective than the monotherapy with 0.1 % tacrolimus ointment. In a recent multicenter, randomized, double-blind, vehicle-controlled trial, 0.1 % tacrolimus ointment was applied in 30 patients with different CLE subtypes and resulted in a significantly higher response rate than the vehicle (Kuhn et al. 2011b). Patients with ACLE, SCLE, and LET showed significant improvement of edematous lesions on all time points comparing 0.1 % tacrolimus ointment with the vehicle, whereas DLE showed the lowest improvement as described in previous case reports.

In an open-label and uncontrolled study, 1 % pimecrolimus cream was applied in 11 patients with different subtypes of CLE twice daily for 3 weeks (overnight occlusion with hydrocolloid dressings), and improvement was observed in all patients (Kreuter et al. 2004). Similar results were seen in 10 patients with DLE treated with 1 % pimecrolimus cream twice daily for 8 weeks (Tlacuilo-Parra et al. 2005). In a recent randomized double-blind pilot study, significant improvement was obtained by 1 % pimecrolimus cream and topical 0.1 % betamethasone 17-valerate cream in 10 patients with moderate to severe facial DLE groups (Barikbin et al. 2009). A decrease of 86 % and 73 % in clinical disease severity score was seen with 1 % pimecrolimus and 0.1 % betamethasone cream, respectively. To date, tacrolimus ointment and pimecrolimus cream are only approved for the use in atopic dermatitis in children and adults. Despite their off-label use, calcineurin inhibitors are recommended for topical application in SLE and CLE, particularly in atrophy-prone areas such as the face.

R-Salbutamol

In 37 patients with DLE, a multicenter, double-blind, randomized, placebo-controlled phase II trial was performed using R-salbutamol for 8 weeks twice daily (Jemec et al. 2009). Scaling/hypertrophy, pain, itching, and allover patient assessment were significantly more efficient in the R-salbutamol-treated group compared with the placebo-treated group. However, phase III trials still need to be performed to approve the efficacy of R-salbutamol in SLE and CLE.

Other Topical Therapies

Topical retinoids, such as tazarotene and tretinoin, have only been applied in single case reports of therapy-refractory hypertrophic CLE or in disseminated DLE (Seiger et al. 1991; Edwards and Burke 1999). In addition, the topical agent 5 % imiquimod cream was applied in one patient with DLE once daily in two cycles over 3 weeks, and imiquimod showed a marked improvement in treating erythema and follicular scaling of the scalp in a patient with DLE (Gerdsen et al. 2002). However, there may be a theoretical risk in some patients of exacerbating cutaneous and systemic diseases, as imiquimod is a toll-like receptor agonist. Physical therapies, such as laser or cryotherapy and dermabrasion, are reported to be successful in single case reports but should only be used in persistent DLE lesions, after risk-benefit analysis and evaluation of other therapeutic options due to the possible induction of Koebner phenomenon (Kuhn et al. 2011c).

Systemic Treatment

Antimalarials

Antimalarials, such as hydroxychloroquine (HCQ), chloroquine (CQ), and mepacrine (synonym: quinacrine), are the first-line therapy for all skin manifestations in SLE and CLE (Kuhn et al. 2010c). However, only one randomized,

placebo-controlled study comparing acitretin with hydroxychloroquine was performed in 1992 (Ruzicka et al. 1992). About 50 % of patients improved when treated with HCQ, and 46 % showed improvement after being treated with acitretin, but more side effects were seen in the acitretin group. HCQ has been suggested to be more compatible than CQ but less effective. This might be due to the fact that especially in earlier case series, CQ was used in higher doses than HCQ; comparative studies are missing. The maximal oral daily doses of HCQ are 6–6.5 mg/kg ideal body weight and 3.5–4 mg/kg ideal body weight for CQ; higher dosages should only be given for a short period of time (Ochsendorf 2010). Oral doses of 100 mg/day mepacrine (one tablet) should not be exceeded. After achieving a good response (within 3–6 months), the dose of mepacrine should be tapered until maintenance doses of one to three tablets a week. In case of adverse reactions, the daily dose may be reduced to 25–50 mg.

Irreversible retinal changes are the most feared side effect of HCQ and CQ treatment. The incidence of retinopathy is related to the maximal daily dosage, which should comply with the ideal body weight of adults and children to avoid this effect (Kuhn et al. 2011c). Due to the toxicity and side effects, it is important to evaluate the visual findings before or during treatment initiation. Acute overdosing happens accidentally, mainly in children, or during an attempted suicide. Therefore, patients have to be informed about the importance of a safe storage of these drugs in order to protect their children. Several studies confirm that smoking interferes with the efficacy of antimalarials (Kreuter et al. 2009), and some mechanisms by which smoking may alter the antimalarial metabolism have been proposed. For example, it has been suggested that nicotine inhibits CQ uptake in cultured cells and blocks the accumulation of antimalarials within lysosomes (Polet 1985). Recent studies investigating the relationship between smoking and the efficacy of antimalarials in CLE patients, however, indicate that cigarette smoking does not have any significant influence on the response to HCQ and CQ (Wahie et al. 2011). Therefore, prospective studies are required to determine whether antimalarials are more likely to benefit current nonsmokers (Dutz and Werth 2011).

Systemic Corticosteroids

Systemic corticosteroids may be indicated for cutaneous manifestations in SLE and CLE despite the well-known side effects such as hyperglycemia and osteoporosis. The application should, however, be limited to patients with highly acute and severe skin lesions or may be given due to the delayed onset of action of antimalarials (Kuhn et al. 2011c). The usual dose of systemic corticosteroids is 0.5–1 mg/kg bodyweight/day over 2–4 weeks followed by tapering of the dose or as a 3-day intravenous (IV) pulse therapy as described in SCLE (Goldberg and Lidsky 1984). In this study, it has been demonstrated that refractory disease improved within 1–2 weeks after IV pulse therapy with 1 g of methylprednisolone on three successive days. Although DLE responds to high doses (>1 mg/kg bodyweight), systemic corticosteroids are usually not indicated in this subtype due to rapid rebound above the Cushing threshold dose.

Methotrexate (MTX)

MTX has been successfully used for the treatment of therapy-refractory CLE (Kuhn et al. 2011d). In SCLE and DLE, it is recommended to apply MTX in a dose of 7.5–25 mg via subcutaneous (sc) injection; to prevent gastrointestinal side effects, folic acid supplementation may additionally be given once 24 h after MTX injection excluding the day of MTX application and the day thereafter (Kuhn et al. 2010c). In a retrospective analysis from 1998, 12 patients with different subtypes of CLE were treated with MTX in a low dose of weekly 10–25 mg (orally or IV) (Boehm et al. 1998). The beneficial effect of MTX for skin lesions was confirmed in a study including 43 patients with different subtypes of recalcitrant CLE (Wenzel et al. 2005). In 98 % of patients, the skin lesions improved with low-dose MTX, administered either orally or IV; a better improvement was observed in patients with SCLE and localized DLE than in disseminated DLE.

In a follow-up study, MTX IV administration was changed to sc application in 15 of the 43 CLE patients with good tolerance and acceptance due to easier and self-administered injection; the efficacy was comparable (Huber et al. 2006).

Retinoids

Retinoids, such as acitretin and isotretinoin, are listed as second-line substances for CLE in the American Academy of Dermatology guidelines due to their relatively innocuous side-effect profile (Drake et al. 1996). These agents have been effectively applied in cases of DLE and SCLE (Bacman et al. 2004). The recommended dose for acitretin and isotretinoin is 0.2–1.0 mg/kg body weight per day. Both retinoids are teratogenic; therefore, effective contraception is essential during and after treatment (isotretinoin, 1 month; acitretin, 2 years). Isotretinoin is preferable in female patients of childbearing potential due to the shorter half-life. Recently, a case report on three patients with different subtypes of CLE who received alitretinoin showed a high efficacy in the treatment of CLE skin lesions (Kuhn et al. 2012). Thus, this agent may be an effective alternative in the treatment of CLE. Most common side effects of retinoids include dryness of skin and mucous membranes (xerophthalmia, xerostomia), less common are gastrointestinal disturbances and skeletal toxicity, as well as muscle pain and arthralgia (Bacman et al. 2004). Treatment with retinoids may cause hyperlipidemia and alter liver function tests, but severe hepatotoxic reactions are only seen in single cases. During treatment with acitretin, reversible hair loss has been reported in up to 50 % of patients.

Dapsone

Dapsone has been demonstrated to be effective in SCLE, LEP, urticarial vasculitis, oral ulcerations, and bullous eruptions complicating SLE (Bacman et al. 2004; Ludgate and Greig 2008). Therapeutic dosages of dapsone range from 25 to 150 (maximum 200) mg/day; it is recommended to start with a low dose and to increase the dose gradually. The lowest effective dose should be applied to minimize possible side effects, such as hemolysis and methemoglobinemia. Prior to treatment, exclusion of glucose-6-phosphate dehydrogenase deficiency is necessary as regular monitoring of blood cells and liver enzymes during treatment with dapsone. Several studies report the efficacy of dapsone in patients with DLE, and single case reports of successful treatment of SCLE with dapsone have also been described in the literature (Kuhn et al. 2011d). In LEP, successful treatment with dapsone was reported in 12 patients published by a Japanese group (Ujiie et al. 2006).

Mycophenolate Mofetil (MMF) and Mycophenolate Sodium (EC-MPS)

Another immunosuppressive drug is MMF, which has been proved to be effective in single case reports of SCLE, DLE, and CHLE. MMF should be administered in doses between 1 and 3 g per day, and the highest efficacy is achieved with 2–3 g per day, but it can take 1–2 months for clinical effects to become visible. In general, MMF is well tolerated by the most patients (Kuhn et al. 2011d). In 2002, four therapy-resistant patients with various skin manifestations of CLE and smoldering systemic involvement resulted in a complete remission within 3 months after starting MMF (Hanjani and Nousari 2002). However, this result could not be confirmed by Pisoni et al. (2005) in five of seven SLE patients with various LE-specific and nonspecific skin manifestations. In a recent nonrandomized, open-pilot study, the enteric-coated form of MMF (EC-MPS) was applied in 10 patients with active SCLE resistant to at least one standard therapy (Kreuter et al. 2007). The treatment resulted in significant improvement of the cutaneous manifestations, while no serious side effects were reported. However, controlled trials with a larger number of patients are required to further assess efficacy and safety of MMF or EC-MPS in SLE and CLE.

Thalidomide and Lenalidomide

In a high number of patients with DLE, thalidomide has been proven to be effective due to the agent's immunomodulatory and anti-inflammatory features. These studies also

demonstrated the high relapse rate and, most importantly, the high risk of potential irreversible polyneuropathy (Kuhn et al. 2011d). Therefore, thalidomide should only be used to treat severe refractory DLE; however, there is a high incidence of neurotoxicity even at low doses (Cuadrado et al. 2005). Thalidomide should only be applied in appropriately selected patients after intensive consideration of the risk-benefit ratio. Moreover, due to its teratogenic effect, this agent should only be prescribed to women of childbearing potential with severe and refractory DLE and if highly effective contraceptive measures are applied (Kuhn et al. 2010c).

Lenalidomide is a structural derivative of thalidomide introduced in 2004. In one recent open-label study, 5 mg of oral lenalidomide daily during the first 6 weeks of treatment led to clinical improvement in 4 of 5 patients with CLE (Braunstein et al. 2011). However, one of the patients subsequently developed symptoms of SLE. The same strict safety measures used for thalidomide have to be applied for lenalidomide, but the risk of neurotoxicity seems to be lower for lenalidomide.

Belimumab

Belimumab belongs to a new class of immunomodulators with a novel mechanism of action, which was approved 2011 for SLE based on two phase III studies (Furie et al. 2011; Navarra et al. 2011). In doses of 1 and 10 mg/kg, belimumab was applied in 1,684 autoantibody-positive patients. Disease activity significantly improved in mucocutaneous organ manifestations as analyzed by the Safety of Estrogens in Lupus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) and the British Isles Lupus Assessment Group score (BILAG) organ domain scores (Manzi et al. 2012). However, a validated skin activity and damage score such as the RCLASI was not applied to assess improvement of skin manifestations in SLE. Therefore, future prospective studies using a validated skin score, such as the RCLASI, are necessary to evaluate the effect of belimumab on mucocutaneous lesions.

Conclusion

SLE patients show a wide variety of skin manifestations and should be recommended to follow instructions regarding methods of protection from sunlight and artificial sources of UV radiation including application of broad-spectrum sunscreens with physical and chemical filters as well as wearing hats and tightly woven clothing. Topical corticosteroids have been proven to be a highly effective treatment for skin lesions in all disease subtypes; a short and intermittent treatment is preferable due to the well-known side effects. Topical calcineurin inhibitors are particularly useful for facial lesions, but the efficacy of these agents still needs to be evaluated in randomized controlled phase III trials. The indication for systemic therapy in CLE is given when skin lesions are widespread, disfiguring, scarring, or refractory to topical agents. Although antimalarials have been available for more than 50 years, these agents still represent the first-line systemic treatment in all CLE subtypes. Second-line treatment includes retinoids, dapsone, MTX, and MMF (EC-MPS). Belimumab has recently demonstrated significant improvement in mucocutaneous manifestations, but further prospective studies using a validated skin score are necessary to assess its efficacy in skin lesions of SLE.

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Cross-References

- ▶ Discoid SLE
- ▶ Immunology of Alopecia in Autoimmune Skin Disease
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Systemic Lupus Erythematosus, Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Albrecht J, Berlin JA, et al. Dermatology position paper on the revision of the 1982 ACR criteria for systemic lupus erythematosus. Lupus. 2004;13(11):839–49.

Arur S, Uche UE, et al. Annexin I is an endogenous ligand that mediates apoptotic cell engulfment. Dev Cell. 2003;4(4):587–98.

Bacman D, Kuhn A, et al. Dapsone and retinoids. In: Kuhn A, Lehmann P, Ruzicka T, editors. Cutaneous lupus erythematosus. Berlin: Springer; 2004. p. 373–90.

Baima B, Sticherling M. Apoptosis in different cutaneous manifestations of lupus erythematosus. Br J Dermatol. 2001;144(5):958–66.

Barikbin B, Givrad S, et al. Pimecrolimus 1 % cream versus betamethasone 17-valerate 0.1 % cream in the treatment of facial discoid lupus erythematosus: a double-blind, randomized pilot study. Clin Exp Dermatol. 2009;34(7):776–80.

Boehm IB, Boehm GA, et al. Management of cutaneous lupus erythematosus with low-dose methotrexate: indication for modulation of inflammatory mechanisms. Rheumatol Int. 1998;18:59–62.

Braunstein I, Goodman NG, et al. Lenalidomide therapy in treatment-refractory cutaneous lupus erythematosus: histologic and circulating leukocyte profile and potential risk of a systemic lupus flare. J Am Acad Dermatol. 2011;66:571–82.

Costner MI, Sontheimer RD, et al. Lupus erythematosus. In: Sontheimer RD, Provost TT, editors. Cutaneous manifestations of rheumatic diseases. Philadelphia: Williams & Wilkins; 2003. p. 15–64.

Cuadrado MJ, Karim Y, et al. Thalidomide for the treatment of resistant cutaneous lupus: efficacy and safety of different therapeutic regimens. Am J Med. 2005;118(3):246–50.

Drake LA, Dinehart SM, et al. Guidelines of care for cutaneous lupus erythematosus. J Am Acad Dermatol. 1996;34:830–6.

Dutz J, Werth VP. Cigarette smoking and response to antimalarials in cutaneous lupus erythematosus patients: evolution of a dogma. J Invest Dermatol. 2011;131(10):1968–70.

Edwards KR, Burke WA. Treatment of localized discoid lupus erythematosus with tazarotene. J Am Acad Dermatol. 1999;41:1049–50.

Furie R, Petri M, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. Arthritis Rheum. 2011;63(12):3918–30.

Gerdsen R, Wenzel J, et al. Successful treatment of chronic discoid lupus erythematosus of the scalp with imiquimod. Dermatology. 2002;205(4):416–8.

Gilliam JN. The cutaneous signs of lupus erythematosus. Cont Educ Fam Phys. 1977;6:34–70.

Goldberg JW, Lidsky MD. Pulse methylprednisolone therapy for persistent subacute cutaneous lupus. Arthritis Rheum. 1984;27(7):837–8.

Hampton PJ, Farr PM, et al. Implication for photosensitive patients of ultraviolet A exposure in vehicles. Br J Dermatol. 2004;151(4):873–6.

Hanjani NM, Nousari CH. Mycophenolate mofetil for the treatment of cutaneous lupus erythematosus with smoldering systemic involvement. Arch Dermatol. 2002;138(12):1616–8.

Hexsel CL, Bangert SD, et al. Current sunscreen issues: 2007 Food and Drug Administration sunscreen labelling recommendations and combination sunscreen/insect repellent products. J Am Acad Dermatol. 2008;59(2):316–23.

Hinz T, Hornung T et al. Lupus tumidus following the lines of Blaschko. Int J Dermatol. 2012 [epub ahead of print].

Huber A, Tuting T, et al. Methotrexate treatment in cutaneous lupus erythematosus: subcutaneous application is as effective as intravenous administration. Br J Dermatol. 2006;155(4):861–2.

Jemec GB, Ullman S, et al. A randomized controlled trial of R-salbutamol for topical treatment of discoid lupus erythematosus. Br J Dermatol. 2009;161(6):1365–70.

Jessop S, Whitelaw DA et al. Drugs for discoid lupus erythematosus. Cochrane Database Syst Rev. 2009; (4): CD002954.

Kretz CC, Norpo M, et al. Anti-annexin 1 antibodies: a new diagnostic marker in the serum of patients with discoid lupus erythematosus. Exp Dermatol. 2010;19(10):919–21.

Kreuter A, Gambichler T, et al. Pimecrolimus 1 % cream for cutaneous lupus erythematosus. J Am Acad Dermatol. 2004;51(3):407–10.

Kreuter A, Tomi NS, et al. Mycophenolate sodium for subacute cutaneous lupus erythematosus resistant to standard therapy. Br J Dermatol. 2007;156(6):1321–7.

Kreuter A, Gaifullina R, et al. Lupus erythematosus tumidus: response to antimalarial treatment in 36 patients with emphasis on smoking. Arch Dermatol. 2009;145(3):244–8.

Kuhn A, Ruzicka T. Classification of cutaneous lupus erythematosus. In: Kuhn A, Lehmann P, Ruzicka T, editors. Cutaneous lupus erythematosus. Heidelberg: Springer; 2004. p. 53–8.

Kuhn A, Richter-Hintz D, et al. Lupus erythematosus tumidus–a neglected subset of cutaneous Lupus erythematosus: report of 40 cases. Arch Dermatol. 2000;136(8):1033–41.

Kuhn A, Sonntag M, et al. Phototesting in lupus erythematosus tumidus-review of 60 patients. Photochem Photobiol. 2001;73(5):532–6.

Kuhn A, Herrmann M, et al. Accumulation of apoptotic cells in the epidermis of patients with cutaneous lupus erythematosus after ultraviolet irradiation. Arthritis Rheum. 2006;54(3):939–50.

Kuhn A, Sticherling M, et al. Clinical manifestations of cutaneous lupus erythematosus. J Dtsch Dermatol Ges. 2007;5(12):1124–37.

Kuhn A, Böhm M, et al. Tissue injury and the skin. In: Lahita R, Buyon J, Koike T, editors. Systemic lupus erythematosus. New York: Elsevier; 2010a. p. 475–90.

Kuhn A, Meuth AM, et al. Revised Cutaneous Lupus Erythematosus Disease Area and Severity Index (RCLASI): a modified outcome instrument for cutaneous lupus erythematosus. Br J Dermatol. 2010b;163(1):83–92.

Kuhn A, Ochsendorf F, et al. Treatment of cutaneous lupus erythematosus. Lupus. 2010c;19(9):1125–36.

Kuhn A, Ruland V, et al. Photosensitivity, phototesting, and photoprotection in cutaneous lupus erythematosus. Lupus. 2010d;19(9):1036–46.

Kuhn A, Gensch K, et al. Photoprotective effects of a broad-spectrum sunscreen in ultraviolet-induced cutaneous lupus erythematosus: a randomized, vehicle-controlled, double-blind study. J Am Acad Dermatol. 2011a;64(1):37–48.

Kuhn A, Gensch K, et al. Efficacy of tacrolimus 0.1 % ointment in cutaneous lupus erythematosus: a multicenter, randomized, double-blind, vehicle-controlled trial. J Am Acad Dermatol. 2011b;65(1):54–64.

Kuhn A, Ruland V, et al. Cutaneous lupus erythematosus: Update of therapeutic options Part I. J Am Acad Dermatol. 2011c;65:e179–93.

Kuhn A, Ruland V, et al. Cutaneous lupus erythematosus: update of therapeutic options part II. J Am Acad Dermatol. 2011d;65(6):e195–213.

Kuhn A, Patsinakidis N, et al. Alitretinoin for cutaneous lupus erythematosus. J Am Acad Dermatol; 2012, e123–6.

Lee-Kirsch MA, Gong M, et al. Familial chilblain lupus, a monogenic form of cutaneous lupus erythematosus, maps to chromosome 3p. Am J Hum Genet. 2006;79(4):731–7.

Lehmann P, Homey B. Clinic and pathophysiology of photosensitivity in lupus erythematosus. Autoimmun Rev. 2009;8(6):456–61.

Liu Z, Davidson A. Taming lupus-a new understanding of pathogenesis is leading to clinical advances. Nat Med. 2012;18(6):871–82.

Ludgate MW, Greig DE. Bullous systemic lupus erythematosus responding to dapsone. Australas J Dermatol. 2008;49(2):91–3.

Madan V, August PJ, et al. Efficacy of topical tacrolimus 0.3 % in clobetasol propionate 0.05 % ointment in therapy-resistant cutaneous lupus erythematosus: a cohort study. Clin Exp Dermatol. 2010;35(1):27–30.

Manzi S, Sanchez-Guerrero J et al. Effects of belimumab, a B lymphocyte stimulator-specific inhibitor, on disease activity across multiple organ domains in patients with systemic lupus erythematosus: combined results from two phase III trials. Ann Rheum Dis. 2012;71(11):1833–8.

Navarra SV, Guzman RM, et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. Lancet. 2011;377(9767):721–31.

Ochsendorf FR. Use of antimalarials in dermatology. J Dtsch Dermatol Ges. 2010;8(10):829–44.

Petri M, Orbai AM, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012;64(8):2677–86.

Pisoni CN, Karim Y, et al. Mycophenolate mofetil and systemic lupus erythematosus: an overview. Lupus. 2005;14(Suppl 1):s9–11.

Polet H. The effects of lysosomotropic amines on protein degradation, migration of nonhistone proteins to the nucleus, and cathepsin D in lymphocytes. J Cell Physiol. 1985;122(3):415–23.

Roenigk Jr HH, Martin JS, et al. Discoid lupus erythematosus. Diagnostic features and evaluation of topical corticosteroid therapy. Cutis. 1980;25(3):281–5.

Ruzicka T, Sommerburg C, et al. Treatment of cutaneous lupus erythematosus with acitretin and hydroxychloroquine. Br J Dermatol. 1992;127(5):513–8.

Schmitt V, Meuth AM, et al. Lupus erythematosus tumidus is a separate subtype of cutaneous lupus erythematosus. Br J Dermatol. 2010;162(1):64–73.

Seiger E, Roland S, et al. Cutaneous lupus treated with topical tretinoin: a case report. Cutis. 1991;47(5):351–5.

Sontheimer RD. Subacute cutaneous lupus erythematosus: 25-year evolution of a prototypic subset (subphenotype) of lupus erythematosus defined by characteristic cutaneous, pathological, immunological, and genetic findings. Autoimmun Rev. 2005;4(5):253–63.

Tan EM, Cohen AS, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1982;25:1271–7.

Ting W, Stone MS, et al. Toxic epidermal necrolysis-like acute cutaneous lupus erythematosus and the spectrum of the acute syndrome of apoptotic pan-epidermolysis (ASAP): a case report, concept review and proposal for new classification of lupus erythematosus vesiculobullous skin lesions. Lupus. 2004;13(12):941–50.

Tlacuilo-Parra A, Guevara-Gutierrez E, et al. Pimecrolimus 1 % cream for the treatment of discoid lupus erythematosus. Rheumatology (Oxford). 2005;44(12):1564–8.

Torchia D, Romanelli P, et al. Erythema multiforme and Stevens-Johnson syndrome/toxic epidermal necrolysis associated with lupus erythematosus. J Am Acad Dermatol. 2012;67(3):417–21.

Tzung TY, Liu YS, et al. Tacrolimus vs. clobetasol propionate in the treatment of facial cutaneous lupus erythematosus: a randomized, double-blind, bilateral comparison study. Br J Dermatol. 2007;156(1):191–2.

Ueki H. Koebner phenomenon in lupus erythematosus with special consideration of clinical findings. Autoimmun Rev. 2005;4(4):219–23.

Ujiie H, Shimizu T, et al. Lupus erythematosus profundus successfully treated with dapsone: review of the literature. Arch Dermatol. 2006;142(3):399–401.

Vassileva S. Bullous systemic lupus erythematosus. Clin Dermatol. 2004;22(2):129–38.

Vila LM, Mayor AM, et al. Association of sunlight exposure and photoprotection measures with clinical outcome in systemic lupus erythematosus. P R Health Sci J. 1999;18:89–94.
Wahie S, Daly AK, et al. Clinical and pharmacogenetic influences on response to hydroxychloroquine in discoid lupus erythematosus: a retrospective cohort study. J Invest Dermatol. 2011;131(10):1981–6.
Wenzel J, Brahler S, et al. Efficacy and safety of methotrexate in recalcitrant cutaneous lupus erythematosus: results of a retrospective study in 43 patients. Br J Dermatol. 2005;153(1):157–62.
Weyd H, Abeler-Dörner L, et al. Annexin A1 on the surface of early apoptotic cells suppresses $CD8^+$ T cell immunity. PLOS ONE 2013;30;8(4):e62449.

Small Molecules Targeted For the Treatment of Rheumatoid Arthritis

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Synonyms

Chemokine receptor inhibitors; JAK-3 inhibitor; Kinase inhibitors; Syk inhibitor

Introduction

Despite the remarkable therapeutic advancements in rheumatoid arthritis (RA) of the past decades, still only a subset of patients responds to available treatments and few go into sustained remission (Breedveld et al. 2006). Hence, the search for drugs with novel mechanisms of action and/or more convenient forms of administration vigorously endures.

Definition

The term *small molecule in RA* designates cellular elements that are involved in the signal transduction, activation, and/or overall function of the immune cells implicated in the pathogenesis of RA. In the pursuit for innovative therapeutic agents for RA, a rising interest has focused in the development of oral inhibitors of these small molecules.

Protein Kinases

Kinases are enzymes that modify proteins by phosphorylation. Through this transfer of phosphate groups, they regulate the majority of intracellular biochemical pathways and signal transduction.

Janus Kinase 3 Inhibition

The Janus kinase (JAK) family of tyrosine kinases includes JAK1, JAK2, JAK3, and TYK2. These proteins are involved in the growth, survival, development, and differentiation of a variety of cells, being critically important for immune and hematopoietic cells in particular. JAK1 and JAK2 are ubiquitously expressed; however, JAK3 is predominant in T-cells, NK cells, and monocytes, and uniquely binds the γ-chain that serves as the common receptor for the cytokine subfamily of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (O'Shea et al. 2004). JAK3 inhibition therefore blocks early cytokine signaling and the resultant STAT activation, abrogating cytokine-dependent gene regulation and subsequent lymphocyte activation (O'Shea et al. 2004). While most immunosuppressive drugs target widely expressed molecules resulting in systemic adverse reactions, the selectivity of JAK3 inhibitors makes them a very attractive therapeutic target.

CP-690,550 (tofacitinib) is a potent oral JAK 3 inhibitor currently completing phase III trials for the treatment of RA and showing promise toward FDA approval in the near future. In a phase IIA trial, 264 patients with active RA with an inadequate response or toxicity to methotrexate (MTX) or tumor necrosis factor (TNF)-inhibitor therapy were randomized to receive placebo vs. CP-690,550 at 5, 15, or 30 mg twice daily for 6 weeks with an additional 6 weeks of follow-up. At week 6, the ACR20 response rates were 70.5 %, 81.2 %, and 76.8 % in the 5, 15, and 30 mg groups, respectively, compared with 29.2 %

in the placebo group ($P < 0.001$). Improvements in disease activity in CP-690,550-treated patients compared with placebo were seen in all treatment groups as early as week 1. ACR50 and ACR70 response rates significantly improved in all treatment groups by week 4 (Kremer et al. 2009). These response rates have been reproduced in phase 2b dose-ranging studies in RA patients on background MTX with outcome assessments at 12 and 24 weeks (Tanaka et al. 2009; Kremer et al. 2012), as well as in a 6 month phase 2b dose-ranging monotherapy trial (Fleischmann et al. 2012). Data, presented in abstract form, from the phase 3 trials are consistent with the phase 2 responses, but the complete final results have yet to be published. The overall frequency of adverse events (AEs) was higher in tofacitinib-treated patients in all the clinical trials compared to placebo (Kremer et al. 2009, 2012; Tanaka et al. 2009; Fleischmann et al. 2012). These included infections (mainly upper respiratory infections, urinary tract infections, and influenza), gastrointestinal disorders (abdominal pain, diarrhea, and nausea), headache, and cytopenias (leukopenia and anemia). Infections were the main serious AEs (SAEs) seen in the CP-690,550-treated arms (Kremer et al. 2009, 2012; Tanaka et al. 2009; Fleischmann et al. 2012).

Spleen Tyrosine Kinase Inhibition

Spleen Tyrosine Kinase (syk) is an intracellular tyrosine kinase isolated in 1991 from a porcine cDNA library (Taniguchi et al. 1991). It is predominantly expressed in hematopoietic cells where it binds phosphorylated tyrosine-based activation motifs (ITAMS) and mediates B-cell receptor (BCR) signaling in B cells; FcγR signaling in mast cells, macrophages, neutrophils, and basophils; and natural killer receptor's activation (Pamuk and Tsokos 2010). It promotes B- and T-cell development and activation, mast cell degranulation, osteoclast function, neutrophil and macrophage phagocytosis, and platelet activation (Pamuk and Tsokos 2010). In addition, syk activity is upregulated in the RA synovium, where it mediates IL-6 and MMP-3 production and TNFα-induced IL-32, highly expressed in rheumatoid synovial fibroblasts (Lindstrom and Robinson 2010; Mun et al. 2009).

Syk inhibition with R406 and its prodrug R788 is being studied in the treatment of RA. In the collagen-induced arthritis animal model, R788/R406 showed significant improvement in arthritis and suppression of bone erosions, pannus formation, and synovitis (Pine et al. 2007). Following the promising preclinical data, 50, 100, and 150 mg twice daily oral doses of R788 (fostamatinib) vs. placebo were tested over 12 weeks in a randomized clinical trial among 189 patients with active RA refractory to MTX therapy (Weinblatt et al. 2008). An ACR20 response at week 12 was achieved in 65 % and 72 % of the 100 and 150 mg groups, respectively, vs. 38 % in the placebo group ($P < 0.001$). ACR50 and ACR70 responses, and DAS28 remission, were significantly different in the 100 and 150 mg groups when compared to placebo, with clinical response seen as early as week 1. Subsequently, 457 patients with active RA despite MTX were randomized to oral R788 100 mg twice daily vs. 150 mg daily vs. placebo for 6 months (Weinblatt et al. 2010). An ACR 20 response was seen in 67 % and 57 % of patients in the 100 and 150 mg groups, respectively, vs. 35 % in the placebo group ($P < 0.001$). ACR50 and ACR70 responses as well as the DAS remission rate were statistically significantly higher in the 100 mg group, and the ACR50 and DAS remission responses were statistically significantly higher in the 150 mg group. Finally, the ACR20 response at 3 months following syk inhibition was tested in 219 RA patients who had failed biologic agents and they were randomized to treatment with oral R788 100 mg twice daily vs. placebo (Genovese et al. 2011a). With an ACR20 response in the R788 group of 38 % vs. 37 % in the placebo arm, this trial showed no difference in the ACR20, ACR50, or ACR70 responses between the R788-treated patients when compared to placebo. The reason for a lack of demonstrable efficacy in patients with inadequate response to prior biologics is unclear.

The most common adverse events seen in the syk-inhibitor arms were a dose-dependent increase in gastrointestinal side effects and

neutropenia. SAEs included infections, dehydration secondary to diarrhea, bladder cancer, and gastritis (Weinblatt et al. 2008, 2010; Genovese et al. 2011a). Moreover, an open-label extension documented three deaths due to septicemia, sudden death, and cerebral hemorrhage; one case of B-cell lymphoma, one cervical carcinoma, and one myocardial infarction were also reported (Weinblatt et al. 2010).

P38 Mitogen-Activated Protein Kinase (MAPK) Inhibition

Mitogen-activated protein kinases (MAPK) are intracellular enzymes that regulate the production of cytokines and metalloproteinases involved in the inflammatory response and tissue injury in RA. Three major groups of MAPKs have been described: MAPKs-extracellular signal-regulated kinase (ERK), c-JUN N-terminal kinase (JNK), and p38 MAPK (Cobb and Goldsmith 1995). Of these, the p38α MAPK isoform has been of particular interest in RA targeted therapy, given its implication in joint destruction in RA (Mbalaviele et al. 2006).

Following promising preclinical results in RA animal models, several clinical trials have evaluated the efficacy of oral p38 MAPK inhibitors (SCIO-460, Pamapinod, VX-702, BMS-582949) (Genovese et al. 2011b; Cohen et al. 2009; Damjanov et al. 2009; Alten et al. 2010 Feb), but to date none have proven to be clinically efficacious. The reason for this is unclear, but further upstream blockade of this pathway has been suggested as likely to be more beneficial.

Other Tyrosine Kinase Inhibitors

Preclinical studies in mouse models of inflammatory arthritis have evaluated other tyrosine kinase inhibitors considered to be of promising potential for the treatment of RA. In this pursuit, imatinib and nilotinib, known selective inhibitors of a set of protein tyrosine kinases including: abelson kinase (Abl), breakpoint cluster region-abelson kinase (Bcr-Abl), stem cell factor receptor (KIT), platelet-derived growth factor receptor (PDGFR), discoidin domain receptor (DDR), and colony stimulating factor-1 receptor (CSF-1R), have been studied and shown to suppress as well as significantly inhibit the progression of arthritis in animal models (Akashi et al. 2011). Efficacy in humans with RA has not yet been tested, and the available evidence is limited to case reports at the time.

Another kinase of interest is Bruton's tyrosine kinase (BTK). It belongs to the Tec kinase family and is critical for B-cell proliferation, differentiation, and signaling. Defects in the BTK gene lead to X-linked agammaglobulinemia and, based on the success of anti-B-cell therapy in RA, BTK inhibitors are being studied in RA (Kim et al. 2011). Both genetic mutation and pharmacological inhibition of BTK have shown to prevent the development of RA-like inflammatory arthritis in animal models, but, to date, no clinical trials have been reported (Xu et al. 2012).

CCR1, CCR2, and CCR5

Chemokines are a family of small proteins that regulate leukocyte recruitment and activation; consequently, they play a major role in the accumulation of these cells at sites of inflammation, such as the RA synovium. The chemokine family is divided into 4 subgroups: C, CC, CXC, and CXXXC (Galligan et al. 2004). Several of these cytokines have been described at high concentrations in RA synovial tissue and fluid where they can interact with receptors on monocytes (Lebre et al. 2011). Of these receptors, CCR1 (main ligands: CCL3/MIP-1α, CCL5/RANTES, CCL7/MCP-3, CCL8/MCP-2), CCR2 (main ligands: CCL2/MCP-1), and CCR5 (main ligands: CCL3/MIP-1α, CCL5/RANTES, CCL7/MCP-3) have been the main focus of chemokine-targeted therapy in RA, aiming to reduce cell migration and resulting synovial inflammation.

Despite promising results in animal models of RA-like inflammatory arthritis, human trials have been unsuccessful for CCR2 and CCR5 blockade (Vergunst et al. 2008; Gerlag et al. 2010; van Kuijk et al. 2010). For CCR1, after a promising trend to improvement in an early proof-of-concept clinical trial (Haringman et al. 2003), several antagonists were developed

(BX471, CP-481,715, MLN3897) (Meyer and St. Clair 2008) and tested in clinical trials; however, these failed to demonstrate a difference in efficacy in the oral CCR1 antagonist plus MTX arm when compared to the methotrexate plus placebo group (Vergunst et al. 2009; Gladue et al. 2010).

Conclusion

As the knowledge of the pathogenesis of RA increases, more therapeutic modalities targeting specific pathways will be pursued. However, balancing the benefits with the potential toxicities of the drugs in question remains the main challenge to overcome. Of all the small molecule inhibitors, JAK 3 inhibition by CP-690,550 appears the most promising at this time and FDA approval seems likely; however, longer-term follow-up is needed to more precisely delineate its safety profile and sustainability of response. Syk inhibition is a novel potential therapy for RA that has promise in patients who have failed MTX, but has not thus far in those who have failed biologic therapies. Moreover, considerable toxicity noted in the phase 2 trials needs to be further balanced with the potential benefits in subsequent long-term studies. MAPK and CCR inhibitors have not proved promising for the treatment of RA, while other kinases such as BTK, imatinib, and nilotinib remain in preclinical phases of investigation for the treatment of RA.

Even with robust efficacy studies and FDA approval of one or more oral small molecule agents, it will remain to be determined where in the RA treatment algorithm, these drugs will best be utilized. Undoubtedly, at first, use will be restricted to 3rd and 4th line status, after failure of agents with a more established efficacy and safety profile. However, upon proof of safety, efficacy, and cost-effectiveness superiority from head-to-head studies in the appropriate clinical context, it is possible that these agents could supplant more established agents and alter the timing and order of the current RA treatment paradigm.

Cross-References

- ▶ Animal Models in Rheumatoid Arthritis
- ▶ Rheumatoid Arthritis, Biologics in its Treatment
- ▶ Rheumatoid Arthritis, Treatment

References

Akashi N, Matsumoto I, Tanaka Y, Inoue A, Yamamoto K, Sumida T, et al. Comparative suppressive effects of tyrosine kinase inhibitors imatinib and nilotinib in models of autoimmune arthritis. Mod Rheumatol. 2011;21:267–75.

Alten RE, Zerbini C, Jeka S, Irazoque F, Khatib F, Caulfield JP, et al. Efficacy and safety of pamapimod in patients with active rheumatoid arthritis receiving stable methotrexate therapy. Ann Rheum Dis. 2010 Feb;69(2):364–7.

Breedveld FC, Weisman MH, van Vollenhoven R, Cohen SB, Pavelka K, Spencer-Green GT, et al. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. Arthritis Rheum. 2006 Jan;54(1):26–37.

Cobb MH, Goldsmith EJ. How MAP kinases are regulated. J Biol Chem. 1995 Jun 23;270(25):14843–6.

Cohen SB, Cheng TT, Chindalore V, Damjanov N, Burgos-Vargas R, Caulfield JP, et al. Evaluation of the efficacy and safety of pamapimod, a p38 MAP kinase inhibitor, in a double-blind, methotrexate-controlled study of patients with active rheumatoid arthritis. Arthritis Rheum. 2009 Feb;60(2):335–44.

Damjanov N, Kauffman RS, Spencer-Green GT. Efficacy, pharmacodynamics, and safety of VX-702, a novel p38 MAPK inhibitor, in rheumatoid arthritis: results of two randomized, double-blind, placebo-controlled clinical studies. Arthritis Rheum. 2009 May;60(5):1232–41.

Fleischmann R, Cutolo M, Genovese MC, Lee EB, Kanik KS, Zwillich SH, et al. Phase 2B dose-ranging monotherapy study of the oral JAK inhibitor CP-690,550 or adalimumab vs placebo in patients with active rheumatoid arthritis with an inadequate response to DMARDs. Arthritis Rheum. 2012 Mar;64(3):617–29.

Galligan CL, Matsuyama W, Matsukawa A, Mizuta H, Hodge DR, Yoshimura T, et al. Up-regulated expression and activation of the orphan chemokine receptor, CCRL2, in rheumatoid arthritis. Arthritis Rheum. 2004 June;50(6):1806–14.

Genovese MC, Kavanaugh A, Weinblatt ME, Peterfy C, DiCarlo J, Magilavy DB, et al. An oral Syk kinase inhibitor in the treatment of rheumatoid arthritis:

a three-month randomized, placebo-controlled, phase II study in patients with active rheumatoid arthritis that did not respond to biologic agents. Arthritis Rheum. 2011a;63(2):337–45.

Genovese MC, Cohen SB, Wofsy D, Weinblatt ME, Firestein GS, Tong SE, et al. A 24-week, randomized, double-blind, placebo-controlled, parallel group study of the efficacy of oral SCIO-469, a p38 mitogen-activated protein kinase inhibitor, in patients with active rheumatoid arthritis. J Rheumatol. 2011b;38(5):846–54.

Gerlag DM, Hollis S, Layton M, Vencovsky J, Szekanecz Z, Tak PP, et al. Preclinical and clinical investigation of a CCR5 antagonist, AZD5672, in patients with rheumatoid arthritis receiving methotrexate. Arthritis Rheum. 2010 Nov;62(11):3154–60.

Gladue RP, Brown MF, Zwillich SH. CCR1 antagonists: what have we learned from clinical trials. Curr Top Med Chem. 2010;10(13):1268–77.

Haringman JJ, Kraan MC, Smeets TJ, Zwinderman KH, Tak PP. Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. Ann Rheum Dis. 2003 Aug;62(8):715–21.

Kim KH, Maderna A, Schnute ME, Hegen M, Mohan S, Wissner A, et al. Imidazo[1,5-a]quinoxalines as irreversible BTK inhibitors for the treatment of rheumatoid arthritis. Bioorg Med Chem Lett. 2011 Nov 1;21(21):6258–63.

Kremer JM, Bloom BJ, Breedveld FC, Coombs JH, Fletcher MP, Zwillich SH, et al. The safety and efficacy of a JAK inhibitor in patients with active rheumatoid arthritis: results of a double-blind, placebo-controlled phase IIa trial of three dosage levels of CP-690,550 versus placebo. Arthritis Rheum. 2009 Jul;60(7):1895–905.

Kremer JM, Cohen S, Wilkinson B, Connell CA, French JL, Zwillich SH, et al. A phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) versus placebo in combination with background methotrexate in patients with active rheumatoid arthritis and an inadequate response to methotrexate alone. Arthritis Rheum. 2012 Apr;64(4):970–81.

Lebre MC, Vergunst CE, Choi IYK, Aarrass S, Oliveira ASF, Tak PP, et al. Why CCR2 and CCR5 blockade failed and why CCR1 blockade might still be effective in the treatment of rheumatoid arthritis. PLoS ONE. 2011;6(7):e21772.

Lindstrom TM, Robinson WH. A multitude of kinases-Which are the best targets in treating rheumatoid arthritis? Rheum Dis Clin North Am. 2010 May;36(2):367–83.

Mbalaviele G, Anderson G, Jones A, De Ciechi P, Settle S, Monahan J, et al. Inhibition of p38 mitogen-activated protein kinase prevents inflammatory bone destruction. J Pharmacol Exp Ther. 2006 Jun;317(3):1044–53.

Meyer CG, St. Clair EW. Promising biologic agents and small chemical compounds. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Rheumatoid Arthritis, Chapter 10. Philadelphia: Mosby Elsevier; 2008. p. 372–378.

Mun SH, Kim JW, Nah SS, Ko NY, Lee JH, Choi WS, et al. Tumor necrosis factor α-induced interleukin-32 is positively regulated via the Syk/protein kinase C/JNK pathway in rheumatoid synovial fibroblasts. Arthritis Rheum. 2009 Mar;60(3):678–85.

O'Shea J, Pesu M, Borie DC, Changelian PS. A new modality for immunosuppression: targeting the Jak/Stat pathway. Nature Rev. 2004 Jul;3(7):555–64.

Pamuk ON, Tsokos GC. Spleen tyrosine kinase inhibition in the treatment of autoimmune, allergic and autoinflammatory diseases. Arthritis Res Ther. 2010;12(6):222.

Pine PR, Chang B, Schoettler N, Banquerigo ML, Wang S, Brahn E, et al. Inflammation and bone erosion are suppressed in models of rheumatoid arthritis following treatment with a novel Syk inhibitor. Clin Immunol. 2007;124:244–57.

Tanaka Y, Suzuki M, Nakamura H et al. The oral JAK inhibitor CP-690,550 in combination with methotrexate is efficacious, safe and well tolerated in Japanese patients with active rheumatoid arthritis with an inadequate response to MTX alone [Abstract at EULAR]. Copenhagen, Denmark; June 10–13, 2009

Taniguchi T, Kobayashi T, Kondo J, Takahashi K, Nakamura H, Yamamura H. Molecular cloning of a porcine gene syk that encodes a 72-kDa protein-tyrosine kinase showing high susceptibility to proteolysis. J Biol Chem. 1991;266:15790–6.

van Kuijk AW, Vergunst CE, Gerlag DM, Bresnihan B, Gomez-Reino JJ, Tak PP, et al. CCR5 blockade in rheumatoid arthritis: a randomized, double-blind, placebo-controlled clinical trial. Ann Rheum Dis. 2010 Nov;69(11):2013–6.

Vergunst CE, Gerlag DM, Lopatinskaya L, Klareskog L, Smith MD, Tak PP, et al. Modulation of CCR2 in rheumatoid arthritis: a double-blind, randomized, placebo-controlled clinical trial. Arthritis Rheum. 2008 Jul;58(7):1931–9.

Vergunst CE, Gerlag DM, von Moltke L, Karol M, Wyant T, Tak PP, et al. MLN3897 plus methotrexate in patients with rheumatoid arthritis: safety, efficacy, pharmacokinetics, and pharmacodynamics of an oral CCR1 antagonist in a phase IIa, double-blind, placebo-controlled, randomized, proof-of-concept study. Arthritis Rheum. 2009 Dec;60(12):3572–81.

Weinblatt ME, Kavanaugh A, Burgos-Vargas R, Dikranian AH, Medrano-Ramirez G, Grossbard E, et al. Treatment of rheumatoid arthritis with a Syk kinase inhibitor: a twelve-week, randomized, placebo-controlled trial. Arthritis Rheum. 2008 Nov;58(11):3309–18.

Weinblatt ME, Kavanaugh A, Genovese MC, Musser TK, Grossbard EB, Magilavy DB. An oral spleen tyrosine kinase (Syk) inhibitor for rheumatoid arthritis. N Engl J Med. 2010 Sep 30;363(14):1303–12.

Xu D, Kim Y, Postelnek J, Vu MD, Hu DQ, Fine JS, et al. RN486, a selective Bruton's tyrosine kinase inhibitor, abrogates immune hypersensitivity responses and arthritis in rodents. J Pharmacol Exp Ther. 2012 Apr;341(1):90–103.

Spectrum of Minimal Change Disease to Focal Segmental Glomerulosclerosis

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Synonyms

Glomerulosclerosis; Nephrotic syndrome; Podocytes; Proteinuria

Definition

Minimal change disease and focal segmental glomerulosclerosis represent two causes of primary glomerular disease. Minimal change disease is typified by heavy proteinuria, normal renal function, and steroid responsiveness. Focal segmental glomerulosclerosis is also characterized by proteinuria but contrasts with minimal change disease in its tendency to progress to kidney failure and exhibit resistance to steroid therapy. This section will compare and contrast the two diseases.

Introduction

Minimal change disease (MCD) and idiopathic focal and segmental glomerulosclerosis (FSGS) represent two common glomerulopathies manifesting clinically with nephrotic range proteinuria. The podocyte may represent a key cell in the development of each disease. MCD and FSGS may result from genetic anomalies, circulating plasma factors altering glomerular permeability, and/or immune dysfunction with production of a permeability factor that affects podocyte function. Most patients with MCD are steroid responsive, and the majority of patients with idiopathic FSGS are steroid resistant. Treatment focuses on remission of proteinuria and preservation of renal function, utilizing both hemodynamic and immunosuppressive therapies. Immunosuppressive treatment regimens for both diseases generally include the use of corticosteroids. Relapsing or steroid-resistant patients may require the use of a second, often cytotoxic agent. Future studies may help define the pathophysiologic relationship between MCD and idiopathic FSGS and give clues to improved approaches to therapy.

The basic functional unit of the kidney is the nephron, composed of the glomerulus (the primary filtering apparatus) and an associated tubule (concentrates and refines the urine). The glomerulus is a network of capillary loops suspended in Bowman's space and covered with epithelial cells or podocytes. The podocyte is a specialized epithelial cell encasing the capillary loop and plays a central role in the protein filtration barrier function of the nephron.

The normal glomerular filtration barrier consists of fenestrated endothelial cells, the glomerular basement membrane (GBM), and the podocyte – which normally exhibits interdigitating foot processes connected by slit diaphragms. The slit diaphragms are major barriers to protein filtration, which is based upon both molecular size and electric charge.

Minimal change disease (MCD) and idiopathic focal and segmental glomerulosclerosis (FSGS) represent two common glomerulopathies associated with podocyte dysfunction. Both diseases are examples of a podocytopathy and manifest clinically with nephrotic range proteinuria (>3 g/day). The podocyte may represent the key cell in the development of nephrotic syndrome, with the extent of disease determined by both the extent of podocyte damage and the number of podocytes per glomerulus. In this regard, damaged podocytes with normal numbers are associated with MCD, whereas podocytopenia is linked to the development of FSGS. A reduction in podocyte number may result in a loss of structural integrity of the glomerulus favoring "outward

bulging" (Jefferson and Shankland 2006) of the GBM, facilitating abutment on and fusion to Bowman's capsule, and the potential to develop synechiae, an early lesion of FSGS (Jefferson and Shankland 2006).

Podocyte damage in MCD is dominated by foot process effacement with an otherwise normal histologic appearance of the glomerulus (Mason and Hoyer 2010). Less commonly mild mesangial hypercellularity or positive immunofluorescence staining for IgM may be present. It is unclear if these findings are a variation of MCD or represent early changes of FSGS (Mason and Hoyer 2010).

MCD and idiopathic FSGS may share some clinical and pathologic features, but the diseases also differ in many aspects. The purpose of this review is to examine areas of clinical and physiologic overlap and independence between the two conditions and illustrate the features encountered in each disease.

Clinical Epidemiology

MCD is the most common cause of the nephrotic syndrome in children, accounting for 90 % cases in those <10 years of age, and about 50 % of cases in older children. In adults, MCD accounts for 10–15 % of primary nephrotic syndrome (Mason and Hoyer 2010). There is some geographic variation, with the incidence in the United States at 27 per million as compared to 1 per million in the United Kingdom (Mason and Hoyer 2010). MCD is more common in Native Americans and South Asians, and rare in African Americans (Mason and Hoyer 2010). The disease may be idiopathic or associated with atopy, lymphoid disorders, or drug therapies (Mason and Hoyer 2010). Clinically, MCD may present with the sudden onset of edema, selective proteinuria, and is very responsive to corticosteroid therapy (Chugh et al. 2012).

FSGS is less common than MCD in the pediatric population, causing about 20–40 % of cases of idiopathic nephrotic syndrome (Eddy and Symons 2003). In contrast, FSGS is the second most common cause of primary kidney disease in adults and accounts for ~40 % of cases of nephrotic syndrome. FSGS has an estimated prevalence of 7 per million (Gbadegesin et al. 2011; Kitiyakara et al. 2004). FSGS is more common in males, and the overall incidence is much higher in African Americans than Caucasians (Kitiyakara et al. 2004). Clinically, FSGS is the most common primary glomerular disorder that leads to ESRD. FSGS may be idiopathic or secondary to other diseases, including HIV nephropathy (HIVAN), obesity, heroin injection, chronic glomerulonephritis, reflux nephropathy, or other forms of renal ablation (Eddy and Symons 2003; Gbadegesin et al. 2011). Familial forms of FSGS are also recognized (Gbadegesin et al. 2011). Idiopathic forms of FSGS and MCD may be the result of circulating permeability factors that may damage the glomerular filtration barrier (McCarthy et al. 2010). Clinically, idiopathic FSGS presents with nonselective proteinuria and often manifests as steroid-resistant nephrotic syndrome with rapid progression to end-stage renal disease (Gbadegesin et al. 2011).

Histopathology

Minimal change disease. On renal biopsy, MCD is characterized by normal-appearing glomeruli by light microscopy. The GBM and glomerular cellularity are normal. In biopsies exhibiting prominent mesangial proliferation, a diagnosis of diffuse mesangial hypercellularity (DMH) may be considered. This is a variant seen more commonly in young children and demonstrating higher rates of steroid resistance. However, remission rates at 1 year are similar to more typical forms of MCD. In adults with hypertension and vascular disease, MCD may coexist with acute tubular injury and thus present with nephrotic syndrome and an impairment of glomerular filtration (manifesting as acute kidney injury with an elevation in serum creatinine level). Immunofluorescence staining is usually negative. Positive staining for IgM or C1q suggests, respectively, the presence of IgM or C1q nephropathy, both often considered variants of MCD. By electron microscopy, the ultrastructural changes are dominated by podocyte foot process

effacement of >75 % of the total examined surface area. Immune deposits are absent.

FSGS (adapted from (Appel and D'Agati 2010; D'Agati et al. 2004)). Histologically, primary and secondary forms of FSGS are characterized by heterogenous morphologies resulting in the need for a subclassification system (D'Agati et al. 2004). In this approach, five histologic variants can be identified: (1) FSGS, not otherwise specified (NOS); (2) FSGS, perihilar variant; (3) FSGS, cellular variant; (4) FSGS, collapsing variant; and (5) FSGS, tip variant.

FSGS NOS is the generic form of FSGS and excludes the other classifications. Pathologically, it is characterized by accumulation of extracellular matrix forming segmental solidifications in any portion of the tuft with glomerular capillary occlusion. Hyalinosis, foam cells, GBM wrinkling, synechiae, and swollen epithelial cells may be present. Unaffected lobules are normal except for podocyte swelling. By immunofluorescence, staining IgM, C3, and occasionally, C1q may be demonstrated. Electron microscopy lacks immune deposits but shows foot process effacement.

The perihilar variant demonstrates perihilar hyalinosis and is common in secondary forms of FSGS associated with adaptive responses. FSGS cellular variant is associated with endocapillary hypercellularity with segmental capillary occlusion from endocapillary hypercellularity. The collapsing variant of FSGS demonstrates at least one glomerulus with global collapse, hypertrophy and hyperplasia of visceral epithelial cells, and extensive tubulointerstitial disease. The collapsing variant is typically associated with HIVAN. The tip variant of FSGS is associated with at least one segmental lesion involving the outer 25 % of the tuft adjacent to the origin of the proximal tubule. This lesion may be diagnosed only in the absence of perihilar or collapsing sclerosis.

Pathogenesis of the Nephrotic Syndrome in MCD and FSGS

Nephrotic syndrome in MCD and FSGS may result from genetic anomalies, circulating plasma factors altering permeability, and/or lymphoid dysfunction with production of a permeability factor that affects podocyte function.

Genetic Diseases. Genetic mutations of the slit diaphragm proteins nephrin or podocin may result in two different forms of FSGS. In this regard, congenital nephrotic syndrome of the Finnish type is the result of mutations in nephrin, whereas defects in podocin, which links the slit diaphragm to the cell cytoskeleton, are associated with familial FSGS (Jefferson and Shankland 2006). Other inherited disorders of the slit diaphragm protein complex include CD-2-associated protein (CD2AP), α-actinin-4, and the transient receptor potential cation channel type 6 (TRPC6) (Jefferson and Shankland 2006).

CD2AP interacts with the T lymphocyte adhesion protein CD2. In the slit diaphragm, CD2AP interfaces with podocin and nephrin to form a protein signaling complex with phosphoinositide 3-OH kinase (Gbadegesin et al. 2011). Clinically, mutations of CD2AP in humans leads to the development of FSGS (Gbadegesin et al. 2011). Alpha-actinin-4 is an actin protein expressed in the podocyte foot process. Mutations of α-actinin-4 are gain of function, appear to interfere with actin filament assembly and disassembly, and are associated with autosomal dominant FSGS (Gbadegesin et al. 2011). TRPC6 is a cation channel associated with mechanosensation and cell growth. Mutations of TRPC6 have been associated with familial FSGS in both children and adults (Gbadegesin et al. 2011).

Genome-wide association studies have suggested that *MYH9* and *APOL1* may be associated with FSGS, HIVAN, and non-diabetic end-stage renal disease in African Americans (Quaggin and George 2011). *APOL1* has emerged as the more likely candidate gene (Quaggin and George 2011) and encodes apolipoprotein L1. *APOL1* and its renal disease risk alleles appear to confer a trait protective against *T. brucei*, the cause of sleeping sickness in sub-Saharan Africa (Genovese et al. 2010). This association may explain the high carrier frequency of *APOL1* in African Americans with renal disease (Genovese et al. 2010). *APOL1* is expressed in podocytes and proximal tubular cells, and its expression was reduced in kidney

biopsies from patients with FSGS and HIVAN. The mechanisms whereby *APOL1* variants may predispose to FSGS remain to be determined.

Other genetic syndromes associated with FSGS include mutations to genes encoding phospholipase C ε (*PLCE1*), Wilms' tumor suppressor protein (*WT1*), LIM/Homeobox (*LXMZB1*), mitochondrial sequences ($tRNA^{Leu}$), coenzyme Q2 homolog prenyltransferase (*COQ2*), and integrin β4 (*ITGB4*). Many of these conditions are also associated with extensive extrarenal manifestations (Woroniecki and Kopp 2007). Several animal models as well as additional monomeric causes of FSGS have been reported (D'Agati 2012) but are beyond the scope of this review.

Circulating Permeability Factors. Circulating permeability factors have been described in association with idiopathic FSGS and include the soluble urokinase receptor suPAR (Wei et al. 2011) and cardiotrophin-like cytokine-1 (CLC-1) (McCarthy et al. 2010).

Circulating suPAR activates the β_3 integrin on the podocyte foot process which can initiate effacement and proteinuria (Wei et al. 2011). Elevated serum levels of suPAR were identified in two-thirds of idiopathic FSGS patients, but not in MCD or other glomerular diseases (Wei et al. 2011). Increases in suPAR before kidney transplantation were also associated with recurrent FSGS following transplant (Wei et al. 2011). The release of suPAR from activated leukocytes (Pliyev 2009) and from the synovial fluid of rheumatoid arthritis patients (Pliyev and Menshikov 2010) may suggest a role for suPAR in linking the immune system to the pathogenesis of FSGS.

CLC-1 is a small protein isolated from the serum or plasma of patients with recurrent FSGS following kidney transplantation (McCarthy et al. 2010). CLC-1 mimics the effect of plasma from FSGS patients in an in vitro assay of glomerular permeability, decreases glomerular nephrin expression, and is present in elevated levels in patients with recurrent FSGS (McCarthy et al. 2010).

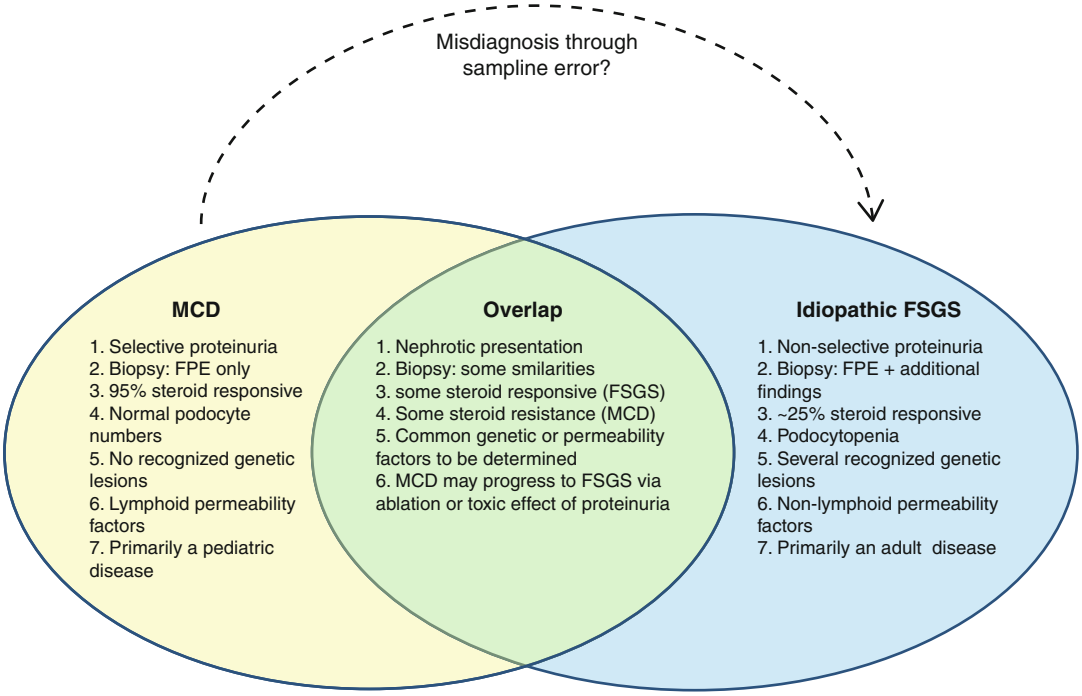
Immune Dysfunction and Circulating Permeability Factors. Nephrotic syndrome has been associated with several primary immunologic disorders, including lymphoma, leukemia, and thymoma (Eddy and Symons 2003; Ishimoto et al. 2011). Variable changes in IL-2, IL-4, and interferon have been reported from subjects with MCD, but findings have been inconsistent (Ishimoto et al. 2011). IL-8 and IL-13 have also been studied as putative mediators of proteinuria in MCD, but the results are inconclusive (Ishimoto et al. 2011).

In 2004, Reiser reported a role for podocyte CD80 as a potential mechanism for proteinuria (Reiser et al. 2004). CD80 is expressed on podocytes, as well as antigen-presenting cells, natural killer cells, and B cells. Expression of CD80 on podocytes results in shape change and proteinuria (Reiser et al. 2004). Urinary CD80 levels are increased in MCD with increased expression on podocytes but not in FSGS (Ishimoto et al. 2011).

The stimuli to increase CD80 podocytes include IL-13 and/or Toll-like receptors (triggered by infection) (Ishimoto et al. 2011). Downregulation of CD80 occurs with CTLA-4, which is secreted and expressed by regulatory T cells (Treg). In this regard, Treg have been shown to be abnormal in MCD. The notion that MCD may represent an imbalance between CD80 induction and CTLA-4 downregulation has been proposed (Shimada et al. 2011). Taken together, these data provide compelling evidence for a pathophysiologic role for CD80 in the development of MCD and suggest it may be a useful biomarker for distinguishing between MCD and FSGS.

Treatment

The goal of treatment for both MCD and FSGS is remission of proteinuria and preservation of renal function. Clinical remission of proteinuria is defined as <1 g/day and is associated with a decreased rate of loss of renal function. Hemodynamic and immunosuppressive therapies alone or in combination may be used to eradicate proteinuria.



Spectrum of Minimal Change Disease to Focal Segmental Glomerulosclerosis, Fig. 1 Comparison of some features of minimal change disease (MCD) to idiopathic focal and segmental glomerulosclerosis (FSGS). The unique characteristics of each disease, and areas of potential overlap are indicated. Areas of overlap support the contention that the two diseases may represent two different spectra of the same disease. *FPE* foot process effacement

Hemodynamic approaches to the management of all forms of glomerular proteinuria include blood pressure control (<130 mmHg systolic), inhibition of the renin-angiotensin-aldosterone system, and/or modification of dietary protein intake to ~0.8 g/kg/day.

Specific immunosuppressive therapies for MCD and FSGS generally include the use of corticosteroids. Children with MCD are typically treated with and have a response rate greater than 95 % (Eddy and Symons 2003). Rates of relapse approach 60 % but may be decreased with longer courses of steroid therapy (Ehrich and Brodehl 1993). Relapses in steroid-dependent cases may require cytotoxic or other therapies to maintain remission. In this regard, 8-week courses of cyclophosphamide or chlorambucil reduced the relapse rate at 12 months compared to prednisone alone, but at 2 years, there was no difference in the relapse rate between the two regimens (Hodson et al. 2008). Prolonged courses of cyclosporine and levamisole may also be useful (Hodson et al. 2008).

The majority of patients with idiopathic FSGS (75–80 %) are steroid resistant and thus do not respond to an 8-week course of high-dose corticosteroids (Eddy and Symons 2003). The patients frequently require consideration of second-line therapy (Eddy and Symons 2003). Cyclosporine significantly increased remission rates in both children and adults (Cattran et al. 2007), but relapses are common after stopping the drug. This must be balanced against the risk of nephrotoxicity (Gbadegesin et al. 2011). A recent NIH trial compared dexamethasone plus mycophenolate mofetil to cyclosporine and found no differences in proteinuria remission rates (Gipson et al. 2011).

Rituximab has also been studied as a second-line approach to the treatment of steroid-resistant

FSGS, but the results did not support its use in the treatment of this disease (Nachman and Glassock 2010). However, in kidney transplant patients at risk for recurrent idiopathic FSGS, rituximab therapy at the time of transplant was associated with a lower incidence of proteinuria and stable renal function following the transplant (Fornoni et al. 2011). In addition, the authors showed that rituximab influences podocyte function by modulating the expression of sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b). Patients with recurrent FSGS showed a reduction in the number of SMPDL-3b positive podocytes. In cultured podocytes, rituximab partially prevented downregulation of SMPDL-3b, cytoskeleton disruption, and podocyte apoptosis that occurred after exposure to serum from patients with recurrent FSGS (Fornoni et al. 2011). These data provide evidence for a direct effect of rituximab on podocyte function, presumably independent of its immunomodulatory effects, and suggest that the full therapeutic potential of the drug remains to be defined.

FSGS as a Continuum of MCD

MCD and idiopathic FSGS represent podocytopathies and have several features in common; however, the two conditions differ in several important ways (Fig. 1). Clinically, MCD "progression" to FSGS or misdiagnosis of MCD for FSGS due to sampling error is likely in some cases (Appel and D'Agati 2010). However, it is clear from the above discussions that MCD and FSGS may result from several independent stimuli, supporting the contention that many cases of FSGS evolve independently of MCD. Ongoing work in the area may serve to resolve this conundrum.

Cross-References

- ▶ Cancer and Nephrotic Syndrome
- ▶ Immune System and Kidney
- ▶ Impact of Recurrent Autoimmune Diseases in Renal Transplant Outcomes
- ▶ Proteinuric Kidney Diseases: Importance of Blood Pressure Control
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Appel G, D'Agati V. Primary and secondary (non-genetic) causes of focal and segmental glomerulosclerosis. In: Floege J, Johnson R, Feehally J, editors. Comprehensive clinical nephrology. 4th ed. St Louis: Elsevier Saunders; 2010. p. 228–40.

Cattran DC, Alexopoulos E, Heering P, Hoyer PF, Johnston A, Meyrier A, Ponticelli C, Saito T, Choukroun G, Nachman P, Praga M, Yoshikawa N. Cyclosporin in idiopathic glomerular disease associated with the nephrotic syndrome: workshop recommendations. Kidney Int. 2007;72(12):1429–47.

Chugh SS, Clement LC, Macé C. New insights into human minimal change disease: lessons from animal models. Am J Kidney Dis. 2012;59(2):284–92.

D'Agati VD. Pathophysiology of focal segmental glomerulosclerosis: new developments. Curr Opin Nephrol Hypertens. 2012;21(3):243–50.

D'Agati VD, Fogo AB, Bruijn JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. Am J Kidney Dis. 2004;43(2):368–82.

Eddy AA, Symons JM. Nephrotic syndrome in childhood. Lancet. 2003;362(9384):629–39.

Ehrich JH, Brodehl J. Long versus standard prednisone therapy for initial treatment of idiopathic nephrotic syndrome in children. Arbeitsgemeinschaft fur Padiatrische Nephrologie. Eur J Pediatr. 1993;152(4):357–61.

Fornoni A, Sageshima J, Wei C, et al. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. Sci Transl Med. 2011;3:85ra46.

Gbadegesin R, Lavin P, Foreman J, Winn M. Pathogenesis and therapy of focal segmental glomerulosclerosis: an update. Pediatr Nephrol. 2011;26(7):1001–15.

Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardy AJ, Hicks PJ, Nelson GW, Vanhollebeke B, Winkler CA, Kopp JB, Pays E, Pollak MR. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science. 2010;329(5993):841–5.

Gipson DS, Trachtman H, Kaskel FJ, Greene TH, Radeva MK, Gassman JJ, Moxey-Mims MM, Hogg RJ, Watkins SL, Fine RN, Hogan SL, Middleton JP, Vehaskari VM, Flynn PA, Powell LM, Vento SM, McMahan JL, Siegel N, D'Agati VD, Friedman AL. Clinical trial of focal segmental glomerulosclerosis in children and young adults. Kidney Int. 2011;80(8):868–78.

Hodson E, Willis N, Craig J. Non-corticosteroid treatment for nephrotic syndrome in children. Cochrane Database Syst Rev. 2008; (1). Art. No.: CD002290. DOI:

10.1002/14651858.CD002290.pub3. Cochrane Database Syst Rev (1):CD002290.
Ishimoto T, Shimada M, Araya CE, Huskey J, Garin EH, Johnson RJ. Minimal change disease: a CD80 podocytopathy? Semin Nephrol. 2011;31(4):320–5.
Jefferson J, Shankland S. Glomerular disease: the podocyte is ready for prime time and may already be center stage. NephSap. 2006;5:331–8.
Kitiyakara C, Eggers P, Kopp JB. Twenty-one-year trend in ESRD due to focal segmental glomerulosclerosis in the United States. Am J Kidney Dis. 2004;44(5):815–25.
Mason P, Hoyer P. Minimal change nephrotic syndrome. In: Floege J, Johnson R, Feehally J, editors. Comprehensive clinical nephrology. 4th ed. St Louis: Elsevier Saunders; 2010. p. 218–27.
McCarthy ET, Sharma M, Savin VJ. Circulating permeability factors in idiopathic nephrotic syndrome and focal segmental glomerulosclerosis. Clin J Am Soc Nephrol. 2010. doi:D – 101271570(– 1555-905X (Electronic)):T – ppublish.
Nachman P, Glassock R. Focal and segmental glomerulosclerosis (acquired and hereditary). NephSap. 2010;9:126–33.
Pliyev BK. Activated human neutrophils rapidly release the chemotactically active D2D3 form of the urokinase-type plasminogen activator receptor (uPAR/CD87). Mol Cell Biochem. 2009;321(1–2):111–22.
Pliyev BK, Menshikov MY. Release of the soluble urokinase-type plasminogen activator receptor (suPAR) by activated neutrophils in rheumatoid arthritis. Inflammation. 2010;33(1):1–9.
Quaggin SE, George Jr AL. Apolipoprotein l1 and the genetic basis for racial disparity in chronic kidney disease. J Am Soc Nephrol. 2011;22(11):1955–8.
Reiser J, von Gersdorff G, Loos M, Oh J, Asanuma K, Giardino L, Rastaldi MP, Calvaresi N, Watanabe H, Schwarz K, Faul C, Kretzler M, Davidson A, Sugimoto H, Kalluri R, Sharpe AH, Kreidberg JA, Mundel P. Induction of B7-1 in podocytes is associated with nephrotic syndrome. J Clin Invest. 2004;113(10):1390–7.
Shimada M, Araya C, Rivard C, Ishimoto T, Johnson RJ, Garin EH. Minimal change disease: a "two-hit" podocyte immune disorder? Pediatric nephrol (Berl, Ger). 2011;26(4):645–9.
Wei C, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, Maigue ID, Karumanchi S, Yap H, Saleem M, Zhang Q, Nikolic B, Chaudhuri A, Daftarian P, Salido E, Torres A, Salifu M, Sarwal M, Schaefer F, Morath C, Schwenger V, Zeier M, Gupta V, Roth D, Rastaldi M, Burke G, Ruiz P, Reiser J. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. 2011. doi:D – 9502015 (– 1546-170X (Electronic)):T – epublish.
Woroniecki RP, Kopp JB. Genetics of focal segmental glomerulosclerosis. Pediatric nephrol (Berl, Ger). 2007;22(5):638–44.

Spondyloarthritis: Ankylosing Spondylitis

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Synonyms

Adalimumab; Biologic drugs; Disease-modifying antirheumatic drugs; DMARDs; Etanercept; Golimumab; Infliximab; Nonsteroidal anti-inflammatory drugs; NSAIDs; Salazopyrin; Spondyloarthritis; Spondyloarthropathy; Sulfasalazine

Definition

The term *ankylosing spondylitis* is derived from the Greek roots *ankylos*, or "bent," and *spondylos*, or "vertebral disc."

Introduction

Spondyloarthritis (SpA) constitutes a relatively common group of arthritides that affect up to 2 % of white individuals and have in common the carriage of the histocompatibility leukocyte antigen (HLA) gene B27. The prototypic form of disease is ankylosing spondylitis (AS), and an overview of the history of AS can be found at www.history-of-ankylosingspondylitis.org. The disease occurs worldwide, but there is marked geographic variation in both the prevalence and phenotypic manifestations. Pathologic hallmarks include inflammation within the sacroiliac joints (sacroiliitis), at specific spinal locations such as vertebral corners and endplates, at the attachment of ligaments and tendons to bone (enthesitis), and at specific extra-articular locations such as the anterior uvea and ascending aorta. Concomitant lesions that may precede the development of musculoskeletal manifestations include

inflammatory bowel disease and psoriasis. A distinguishing feature among other forms of arthritis is the propensity to develop ankylosis, particularly in the sacroiliac joints, intervertebral discs, and facet joints. There is a predilection for males, and disease onset is typically in the third and fourth decades of life although it may present in juveniles, either with asymmetric involvement of large joints in the lower limbs or a syndrome of enthesopathy and involvement of the tarsal joints. Genetic studies have demonstrated additional genes beyond B27 that modify susceptibility to disease. Radiographic sacroiliitis is considered a hallmark in AS and is found in more than 90 % of patients, but in early disease, magnetic resonance imaging (MRI) is more sensitive and shows inflammation in the sacroiliac joints. Management of the disease is still focused on physiotherapeutic modalities to maintain spinal mobility, but new anti-inflammatory agents have been developed that target key inflammatory mediators.

Spondyloarthritis: Ankylosing Spondylitis, Table 1 Modified New York criteria for Ankylosing Spondylitis

Criteria
1. Low back pain of at least 3 months' duration improved by exercise and not relieved by rest
2. Limitation of lumbar spine in sagittal and frontal planes
3. Chest expansion decreased relative to normal values for age and sex
4. Bilateral sacroiliitis grade 2–4
5. Unilateral sacroiliitis grade 3 or 4
Definite ankylosing spondylitis
Unilateral grade 3 or 4, or bilateral grade 2–4 sacroiliitis and any clinical criterion

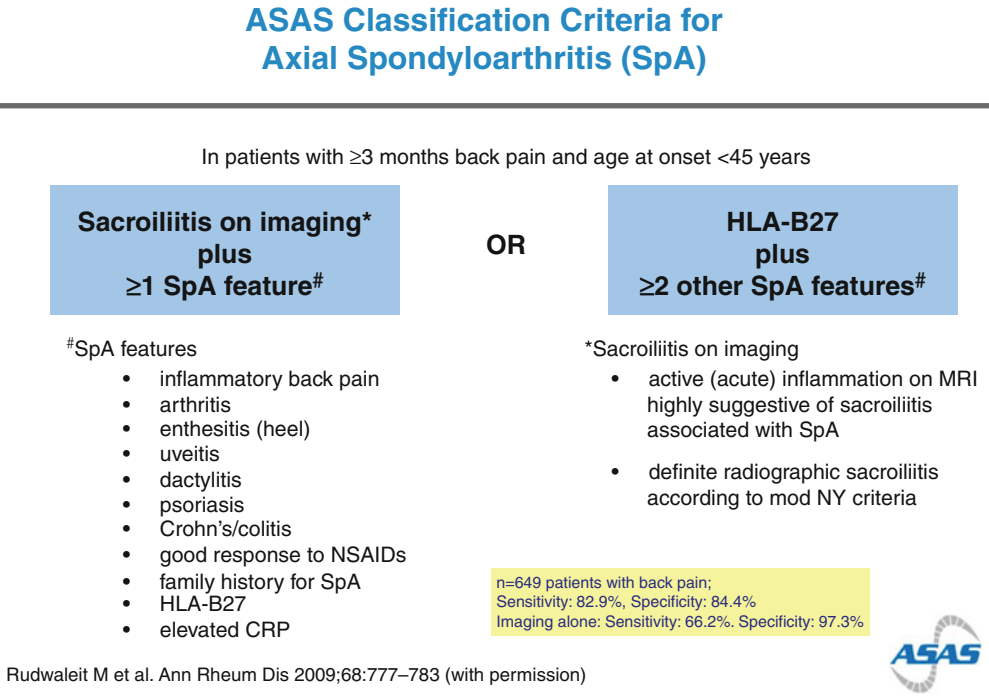
Classification

Radiographic sacroiliitis is considered the primary feature of disease required for establishing a diagnosis and, together with clinical criteria such as the presence of inflammatory back pain and limitation of spinal mobility, is a component of the modified New York criteria. Until recently, these were the primary criteria used to classify AS (Table 1) (van der Linden et al. 1984). Because sacroiliitis may require several years of follow-up before becoming evident on radiography, reliance on these criteria may result in diagnostic delay. It has been shown that sacroiliitis may be evident on MRI prior to the development of radiographic changes, and these patients have been defined as having non-radiographic axial spondyloarthritis (nr-axSpA). In order to combine all patients under the name of a single disease entity, there is now international consensus that the term *axial spondyloarthritis* should be used as the preferred terminology for patients with predominantly spinal disease, and this would include patients with both non-radiographic AS and classical AS according to the modified New York criteria. The Assessments in Ankylosing Spondylitis International Society (ASAS) classification criteria state that a patient can be classified as having axial SpA if there is chronic back pain and age at onset before age 45 together with sacroiliitis on imaging (radiographs or MRI) plus at least one further clinical feature of SpA. In the absence of sacroiliitis on imaging, the diagnosis can be established if the *HLA-B27* is positive and at least two further spondyloarthritis features are present (Table 2) (Rudwaleit et al. 2009). Patients may also present with predominantly peripheral inflammation, and the term for classifying these patients is *peripheral spondyloarthritis*. The ASAS classification criteria state that a patient can be classified as having peripheral SpA in the presence of arthritis and/or enthesitis and/or dactylitis plus (A) one or more of the following parameters, that is, psoriasis, inflammatory bowel disease, preceding infection, HLA-B27, acute anterior uveitis, and sacroiliitis on imaging, or (B) two or more other parameters, that is, arthritis, enthesitis, dactylitis, inflammatory back pain in the past, and family history of SpA (Table 3) (Rudwaleit et al. 2011).

Epidemiology

The prevalence of AS closely parallels the frequency of *HLA-B27*. The estimated prevalence

Spondyloarthritis: Ankylosing Spondylitis, Table 2 ASAS classification criteria for axial spondyloarthritis (SpA)



rate of AS as defined by the modified New York criteria ranges from 68 to 197 per 100,000 but has been estimated at 0.9–1.4 % of the US population and 2.7 million affected persons based on other disease classification criteria and the US National Health and Nutrition Examination Survey (Reveille et al. 2012). AS develops in about 2–5 % of *HLA-B27*-positive adults, and the disease is much more common among *HLA-B27*-positive first-degree relatives of *HLA-B27*-positive AS patients so that 10–30 % of them have signs or symptoms of AS. There are race-related differences in prevalence which may reflect differences in the frequency of *HLA-B27*. For example, AS and *HLA-B27* are nearly absent in African blacks and Japanese.

Pathology

Joint disease in AS most commonly presents as inflammation of the sacroiliac joints. Detailed histopathological studies in early disease are limited but describe granulation tissue beneath the bony endplate eroding through the bone and overlying cartilage into the joint cavity; synovial hypertrophy; infiltration of the subsynovium by macrophages, lymphocytes, and plasma cells; and extension of granulation tissue over the cartilage surface. More advanced disease is highlighted by the presence of many osteoclasts, erosion of bone, obliteration of the synovial joint space by cartilage fusion, and the development of sclerosis in para-articular bone. These lesions account for the classical appearance of joint widening and juxta-articular bone sclerosis observed on radiographic images (Fig. 1). Active degradation of calcified cartilage by chondroclasts and synthesis of endochondral bone by osteoblasts eventually lead to complete replacement of articular cartilage by mature trabecular bone and complete ankylosis of the joint. Immunohistology together with in situ hybridization for mRNA of needle biopsies conducted

Spondyloarthritis: Ankylosing Spondylitis, Table 3 ASAS classification criteria for peripheral spondyloarthritis (SpA)

ASAS Classification Criteria for Peripheral Spondyloarthritis (SpA)

Arthritis or enthesitis or dactylitis plus

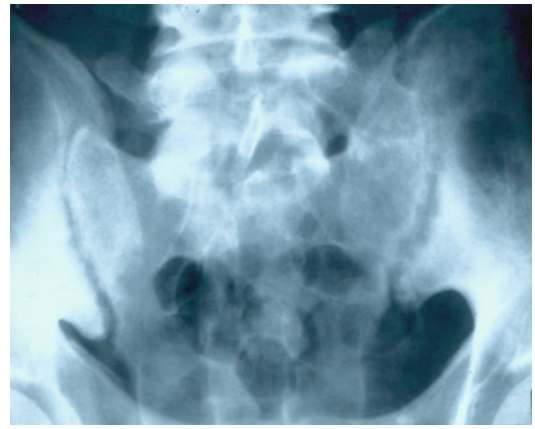
≥ 1 SpA feature	OR	≥ 2 other SpA features
• uveitis • psoriasis • Crohn's/colitis • preceding infection • HLA-B27 • sacroiliitis on imaging		• arthritis • enthesitis • dactylitis • inflammatory back pain (ever) • family history for SpA

Sensitivity: 77.8%, Specificity: 82.2%; n=266

Rudwaleit M et al. Ann Rheum Dis 2011;70:25–31 (with permission)

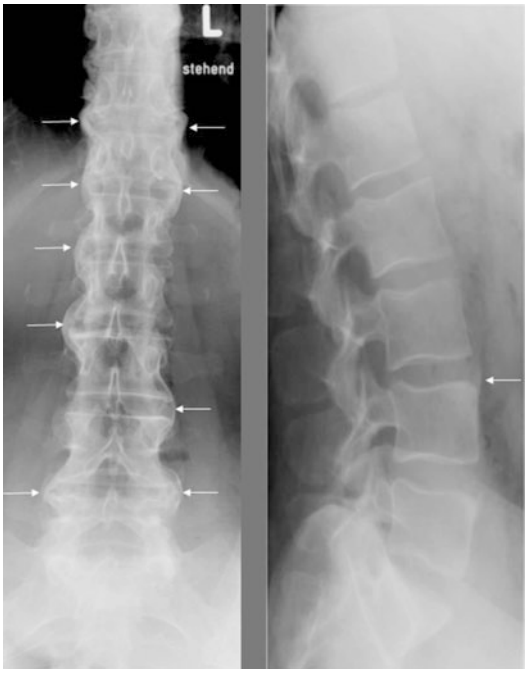
with the guidance of computed tomography has demonstrated dense cellular infiltrates of T lymphocytes and macrophages expressing tumor necrosis factor alpha (TNF-α) (Braun et al. 1995).

In the majority of patients, there is progression of disease to involve the spine with a predilection for involvement of the apophyseal joints, the intervertebral discs, the costovertebral and costotransverse joints, and the extra-articular spinal ligaments. Early inflammation of the apophyseal joints is located at the bony attachment of the joint capsule and is accompanied by a proliferative synovitis with an increase in the number of fibroblasts, small vessels, lymphocytes, plasma cells, and macrophages. This granulation tissue leads to destruction of the capsular insertion and adjacent bone. Ultimately the joint undergoes central cartilage fusion and peripheral ossification leading to ankylosis. Immunohistological analysis of apophyseal joints has shown subchondral lymphocyte infiltrates with CD4+ and CD8+ T cells together with hypervascularization and foci of CD68+ osteoclastic cells.



Spondyloarthritis: Ankylosing Spondylitis, Fig. 1 Pelvic radiograph illustration erosion, pseudo-widening of joints space, and subchondral sclerosis typical of sacroiliitis

At intervertebral disc locations, granulation tissue appears at the attachment of the annulus fibrosus to bone anywhere on the circumference of the vertebra. Cellular infiltration is composed of lymphocytes, plasma cells, and macrophages



Spondyloarthritis: Ankylosing Spondylitis, Fig. 2 New bone formation bridging intervertebral disc space on anteroposterior lumbar radiograph (**a**) and along outer fibers of annulus fibrosus visible as syndesmophyte on lateral lumbar radiograph (**b**)

which results in destruction of the vertebral rim followed by bone proliferation in the adjacent trabecular bone which grows in the direction of the annulus fibrosus, perpendicular to the vertebral axis. As the destruction of the annulus proceeds, ossification extends across the vertical length of the disc and becomes visible on X-ray as a syndesmophyte leading to complete bony ankylosis of adjacent vertebrae as the syndesmophytes encroaching from each vertebra eventually fuse (Fig. 2). This process extends along the length of the vertebral column leading to the appearance of a "bamboo spine." Involvement of the thoracic spine often leads to the development of kyphosis. Vertebral osteoporosis is common and is related to disease activity. It reflects the release of proinflammatory cytokines that promote osteoclastic activity and bone resorption and is associated with an increased risk of vertebral fracture despite the concomitant presence of ankylosis.

Extraspinal lesions can be broadly classified into lesions affecting peripheral entheses, lesions affecting synovial joints such as the hips and knees, and lesions affecting synchondrotic joints such as the manubriosternal joint and symphysis pubis. Entheses affected in AS tend to be those that are rich in fibrocartilage although inflammation within the soft tissue tends to be rather sparse and confined to chronic inflammatory cells such as lymphocytes, macrophages, and plasma cells. Inflammation is prominent within the adjacent bone marrow at the insertion of the ligament, tendon, or joint capsule. Immunohistology shows increased cellular infiltration in the bone marrow with CD8+ T cells and osteoclasts. Synovial joints are affected in about a third of patients with AS, particularly the hips, shoulders, and knees. Immunohistology shows osteoclastic vasoproliferative lesions, while at later stages, there are fibrosis, chondroid metaplasia, and ossification across the joint space and in the joint capsule.

The occurrence of inflammatory lesions in the anterior uvea, aorta, and lungs is well established in AS. Involvement of the aorta is characterized by distinctive fibrous scarring limited to the aortic wall behind and immediately above the sinuses of Valsalva together with dense adventitial scarring extending below the base of the aortic valve to form a characteristic subvalvular ridge. This fibrous tissue may extend into the base of the anterior mitral valve leaflet and may lead to mitral regurgitation. Further extension of this fibrous tissue may also involve the intraventricular septum leading to conduction disturbances. Microscopically there is granulation tissue with accumulation of lymphocytes and plasma cells around small blood vessels in the adventitia followed by fibrosis. Involvement of the anterior uvea is characterized by acute infiltration with inflammatory cells in the iris and ciliary body with accumulation in the aqueous humor. Involvement of the lungs is characterized by apical interalveolar fibrosis or chronic fibrosing pneumonia with lymphocytic infiltration, dilated bronchi, and bullae. A more advanced finding is dense fibrosis, bronchiectasis, and the formation of cavities.

Pathogenesis

The primary role of genetic factors has been known since the first report of an association between AS and the *HLA-B27* gene. More recent data points to an important role for additional genetics factors associated with inflammatory bowel disease and psoriasis and the role of innate immunity.

Genetic Factors

1. *HLA-B27*. Familial aggregation of AS has been recognized for many years, and twin studies have described disease concordance of 75 % in identical twins as compared to 13 % in nonidentical twins.. The risk for AS in *HLA-B27*-positive individuals is in the order of 2–5 %, and 90–95 % of Caucasians with AS are *HLA-B27* positive, but it has been estimated that only about 20 % of the entire genetic risk is due to *HLA-B27*. Only 60–80 % of those individuals with concomitant psoriasis or inflammatory bowel disease carry *HLA-B27*. Of the 50 or so known subtypes of HLA-B27, definite associations have been established with *B*2705* and *B*2702*, the most common subtypes in Caucasians, and *B*2704*, the predominant subtype in Asia among the Chinese and Japanese. Subtypes *B*2706*, the commonest subtype in other Asians, and *B*2709*, primarily observed among Sardinians, do not seem to be associated with AS.

   The main function of HLA class I molecules such as HLA-B27 is to present peptides to cytotoxic T cells. Determination of the crystal structure of *B*2705* as well as characterization of its peptide-binding properties has shown that it has a peptide-binding groove that is conserved between the various *HLA-B27* subtypes but differs from most other *HLA B* molecules. In addition, most naturally bound peptides eluted from *HLA-B27* share an arginine at position 2 that interacts with a glutamine residue in the peptide-binding groove. These observations suggest that the peptide-binding groove of HLA-B27 may convey specificity for the binding of a putative arthritogenic peptide(s) that may elicit T-cell autoreactivity. This forms the basis for one of the main hypotheses addressing the pathophysiological role of *HLA-B27*: cytotoxic T-cell autoreactivity is induced in the course of T-cell-mediated defense to certain bacteria following presentation by *HLA-B27* of an arthritogenic self-peptide(s), perhaps derived from joint and/or entheseal cartilage, in a process that could involve cross-reactivity with bacteria-derived peptides. This is an attractive hypothesis because it conforms to observations demonstrating differing *HLA-B27* subtype associations with disease that could reflect differences in peptide-binding specificities. However, efforts to identify a disease-associated *HLA-B27*-restricted CD8 T-cell response have met with only occasional success, and as yet there have been no examples of cross-reactive CD8 T-cell responses to exogenous antigens demonstrating molecular mimicry with joint antigens. This hypothesis is not consistent with the observation that *HLA-B27* transgenic rats develop severe disease resembling AS even in the absence of CD8+ T cells (Hammer et al. 1990; Taurog et al. 2009).

   A second hypothesis has proposed that peptides derived from the *HLA-B27* molecule itself may be "arthritogenic" when presented to cytotoxic T cells or on class II molecules to $CD4^+$ T cells. A third hypothesis proposes that the *HLA-B27* molecule has additional specific biological properties unrelated to antigen presentation such as an increased propensity to misfold and to form dimers which in turn leads to inflammation on exposure to pathophysiological stimuli. *HLA-B27* may form dimers and multimers through the formation of disulfide links after oxidation of the cysteine residue at position 69, and this has been reported in AS. Binding of these homodimers to macrophages may lead to induction of proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), and they can also trigger direct activation of natural killer (NK) cells through recognition via killer cell immunoglobulin-like

receptors (KIR). Normal folding of the *HLA-B27* molecule is dependent on its association with β_2-microglobulin and peptide. Mutational and peptide-binding analyses of the *HLA-B27* peptide-binding pocket suggest that the propensity of *HLA-B27* to misfold is dependent on its unique structural properties. The assembly of *HLA-B27* into peptide complexes in human *HLA-B27* transgenic rats is indeed slow, leading to misfolding and binding to the binding immunoglobulin protein (BiP) chaperone in the endoplasmic reticulum. BiP is normally bound to certain effector molecules such as activating transcription factor 6 (ATF6), serine/threonine-protein kinase/endoribonuclease (IRE1), and protein kinase RNA-like endoplasmic reticulum kinase (PERK) that trigger stress responses within the cell. Dissociation of BiP from these effector molecules leads to activation of a variety of genes such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) that in turn activate various proinflammatory cytokines (e.g., interleukin-1 and TNF-α). Increased expression of BiP has been reported in synovial fluid cells from patients with AS.

2. *Non-B27* HLA Genes. Population studies and examination of multiplex families have implicated *HLA B60* in risk for disease in *HLA-B27*-positive white individuals, though this is not evident in non-Caucasians. An increased risk associated with *HLA B39* has been observed in the Japanese where the prevalence of *HLA-B27* is low. *HLA B39* shares the same amino acid residues that make up the B pocket in *HLA-B27* and is capable of binding the same peptides.
3. *Non-HLA Genes*. The increased disease concordance of 75 % in identical twins versus 27 % in *HLA-B27* concordant dizygotic twins as well as the increased risk for disease in *HLA-B27*-positive first-degree relatives of AS patients (10–20 %) versus *HLA-B27*-positive individuals in the general population (2–5 %) clearly points to an important contribution from non-HLA genes. Case–control studies have described an association with polymorphism in the cytochrome P450 CYP2D6 gene located on the long arm of chromosome 22. This gene is involved in the metabolism of a variety of drugs and chemicals though the mechanism underlying this association is not understood. Recent genome-wide association studies of AS have identified several novel AS-associated polymorphisms in non-HLA genes (Burton et al. 2007; Reveille et al. 2010). A consistent association has been described with polymorphism in the endoplasmic reticulum aminopeptidase-1 gene (ERAP-1), which encodes an enzyme involved in the trimming of peptides for loading onto MHC molecules. This gene also interacts with *HLA-B27* in predisposing to AS, and polymorphism in this gene is also associated with susceptibility to psoriasis. Disease association has also been described with polymorphisms in the interleukin-23 receptor (IL23R) gene, a locus also associated with susceptibility to Crohn's disease. IL23 is a major regulator of interleukin-17 (IL17) which is expressed in myeloid cells in the spine of patients with AS. In addition, genome-wide association studies have implicated several additional genes with potential roles in inflammation such as the tumor necrosis factor (TNF) receptor 1 (TNFR1), the signaling molecule TNFR1-associated death domain protein (TRADD), the TNF superfamily cytokine TNFSF15, IL-1A and the IL-1 receptor 2 (IL-1R2), the vascular morphogenesis protein gene anthrax toxin receptor 2 (ANTXR2), and the innate immune receptor caspase recruitment domain family member 9 (CARD9). In particular, the associations with genes involved in TNF signaling point to the importance of this cytokine. A TNF-overexpressing mouse model also develops sacroiliitis.

Bacteria and Intestinal Inflammation

Neither *HLA-B27* transgenic rats nor *HLA-B27* transgenic mice develop either intestinal inflammation or peripheral arthritis if maintained in a germ-free environment. Endoscopic studies in patients with AS have demonstrated the presence

of intestinal inflammation in up to 60 % of patients, particularly those with active peripheral joint disease. Prospective assessment of patients who initially present with peripheral joint disease has shown that intestinal inflammation increases the likelihood of progression to axial disease. Altered small intestinal permeability has been described in AS patients and asymptomatic first-degree relatives as well as increased numbers of B cells expressing CD45Ro+, a marker for memory cells, consistent with exposure to luminally derived antigens. Furthermore, identical T-cell expansions in the colonic mucosa and synovium have been reported in AS associated with colitis. Antigen-presenting cells in both intestinal mucosa and synovium express the CD163 scavenger receptor and possess the capacity to secrete TNF-α and IL1 in response to bacterial lipopolysaccharide. Furthermore, this subset of macrophages is increased in AS compared to RA synovium, and levels of CD163 in synovial fluid correlate with disease activity. An abnormal reactivity of innate immune cells such as macrophages, neutrophils, and mast cells has also been proposed on the basis of genetic associations with CARD9 and the IL-1 pathway, and histological studies demonstrating increased infiltration with innate immune cells, but not T, B, or dendritic cells in the peripheral joint and gut of patients with AS. In particular, increased IL17 expression has been noted in cells of the myeloid lineage rather than T cells.

The Link Between Inflammation and Ankylosis

Demonstration of an association between inflammation and spinal ankylosis has been challenging but is of crucial importance because of its implications for treatment. Until the advent of MRI, prospective study was not possible due to inaccessibility of tissue. Recent studies using MRI have described an association between the presence of inflammation at vertebral corners and the subsequent development of syndesmophytes at the corresponding vertebral corners on radiography (Maksymowych et al. 2009). Although reports have also highlighted the development of new syndesmophytes where the baseline MRI shows no inflammation, MRI has limited sensitivity for detection of spinal inflammation that is clearly evident on histopathology. It may be possible that very early inflammatory lesions resolve completely without sequelae if anti-inflammatory therapy with TNF blockers is introduced early at a time when bone formation pathways have not been triggered. Osteoproliferation is dependent on endochondral bone formation which is regulated by several molecular pathways including bone morphogenetic protein (BMP) and wingless protein (Wnt). Dickkopf-1 is a major regulator of Wnt signaling, and its function appears to be impaired in AS.

Clinical Manifestations

The most common presenting feature is lower back pain associated with morning stiffness that is alleviated by activity and exacerbated by rest. The back pain may be particularly prominent at night resulting in nocturnal awakening in the second half of the night. Spinal involvement often ascends the spine but may also skip regions so that occasionally there is severe neck involvement with minimal thoracic disease. On examination there may be limitation of motion of the lumbar spine as elicited by forward flexion, hyperextension, or lateral flexion. Early loss of the normal lumbar lordosis is often the first sign and is easily assessed on inspection. A major complaint is the limitation of ability to drive because of impairment in cervical rotation. Patients may experience chest pain accentuated by coughing or sneezing due to involvement of the costovertebral and costotransverse joints. Mild to moderate reduction of chest expansion is often detectable. Enthesitis may present as pain and swelling around the heel, around the insertion of ligaments and tendons in the patella, at the greater trochanter, and at the insertion of the supraspinatus into the greater tuberosity of the humerus. Shoulder and hip pain is evident in about 20 % of patients.

Extraspinal features include acute anterior uveitis in about 25–30 % of patients. The onset

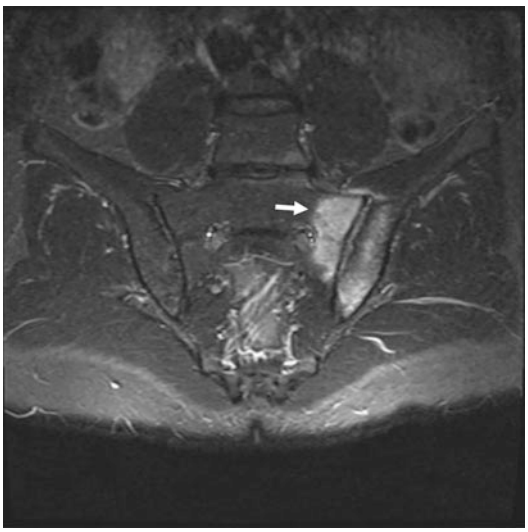
of eye inflammation is usually acute and typically unilateral, but the attacks may alternate. The eye is red and painful, with visual impairment. Photophobia and increased lacrimation may be present. Most attacks subside in 4–8 weeks without sequelae if early treatment is provided. Cardiac involvement includes ascending aortitis, aortic valve incompetence, conduction abnormalities, cardiomegaly, and pericarditis. Cardiac conduction disturbances are seen with increasing frequency with the passage of time. There is an increased incidence of cardiovascular events in patients with AS (Szabo et al. 2011). Lung involvement is characterized by slowly progressive fibrosis of the upper lobes of the lungs and is associated with cough, dyspnea, and hemoptysis.

Neurologic complications of AS can be caused by fracture, instability, compression, or inflammation. Cervical fractures may occur in road traffic accidents. Atlantoaxial joint subluxation, atlantooccipital subluxation, and upward subluxation of the axis may occur in AS as a consequence of instability resulting from the inflammatory process. The cauda equina syndrome is a serious complication of long-standing AS associated with arachnoiditis. The syndrome affects lumbosacral nerve roots giving rise to pain and sensory loss, urinary and fecal incontinence, impotence, saddle anesthesia, and occasionally muscle weakness. Renal manifestations include IgA nephropathy and amyloidosis, especially in very active and long-standing disease. Renal complications such as hypertensive renal disease and renal failure may be associated with long-term use of nonsteroidal anti-inflammatory agents (NSAIDS). The prevalence of symptomatic osteoporotic spinal fractures is increased in AS. Osteoporotic deformities of the thoracic spine contribute significantly to the kyphotic posture.

Diagnosis

In early disease, the presence of back pain with inflammatory features on historical enquiry may be the only helpful diagnostic features. Physical signs are often absent and elevated acute-phase reactants, C-reactive protein, and erythrocyte sedimentation rate are increased in about half of patients. A mild normochromic anemia may be present in 15 % of patients. Elevation of serum alkaline phosphatase (derived primarily from bone) as well as serum IgA may occur.

Physical signs are often absent, while radiography may not reveal evidence of sacroiliitis for several years. The plain anteroposterior view of the pelvis is usually adequate for diagnostic purposes. In young patients with inflammatory chronic back pain, a positive *HLA-B27* test increases the likelihood of having AS, particularly if radiography of the SI joints does not provide conclusive results. MRI shows features of sacroiliitis sooner than radiography and typically shows evidence of bone marrow edema associated with inflammation in the subchondral bone marrow (Fig. 3) (Weber et al. 2011). Consequently, it is now an acceptable imaging criterion for the diagnosis of spondyloarthritis and is especially useful when the history points to inflammatory back pain, the pelvic radiograph is normal or equivocal, and the patient is positive for *HLA-B27*.



Spondyloarthritis: Ankylosing Spondylitis, Fig. 3 Short tau inversion recovery MRI sequence of AS patient demonstrating bone marrow edema in left sacroiliac joint

Prognosis

The course of AS is highly variable, characterized by spontaneous remissions and exacerbations.

Life expectancy is somewhat reduced, particularly after 10 years of disease, with an increased risk of dying due to complications of the disease such as amyloidosis and spinal fractures, as well as cardiovascular, gastrointestinal, and renal disease. Withdrawal from work varies from 10 % after 10 years of disease duration to 30 % after 20 years. Most of the loss of function occurs within the first 10 years and is associated with the presence of peripheral arthritis, spinal radiographic changes, and development of a bamboo spine. Radiographic evidence of spinal involvement, especially the presence of syndesmophytes, is the primary factor that independently predicts further radiographic progression.

Management

Several systematic reviews have recently been published outlining evidence-based approaches to treatment (Zochling et al. 2006; Maksymowych 2006). The treatment objectives are to relieve pain, stiffness, and fatigue, and to maintain good posture and good physical and psychosocial functioning. No drug is currently available that significantly influences the course of spinal disease and retards the process of ossification. Patient education improves self-efficacy and exercise compliance. Patients should avoid vigorous or contact sports if the spine has become fused or osteoporotic because of increased susceptibility to fracture. A program of supervised group physiotherapy is superior to individualized programs in improving thoracolumbar mobility and fitness. Intensive inpatient physiotherapy may also be useful resulting in marked improvement in function and spinal mobility. A Cochrane review concluded that a home exercise program is better than no intervention, supervised group physiotherapy is better than home exercises, and that combined inpatient spa-exercise therapy followed by supervised outpatient weekly group physiotherapy is better than weekly group physiotherapy alone (Dagfinrud et al. 2004).

Many nonsteroidal anti-inflammatory agents (NSAIDs) are effective in patients with AS, and no NSAID has documented superiority in terms of efficacy. Selective cyclooxygenase-2 (COX-2) inhibitors have similar efficacy to conventional NSAIDs. Up to 2 weeks may be required to demonstrate maximal symptomatic benefit from an NSAID. When given for prolonged periods of up to a year, there may be improvement in spinal mobility and acute-phase reactants. There is increasing evidence that continuous use of NSAID may be associated with less spinal fusion, especially in patients with significant inflammatory manifestations of disease.

Disease-modifying agents typically used in rheumatoid arthritis have also been assessed in AS. Most data has focused on the use of sulfasalazine and has demonstrated a beneficial impact of treatment in those patients with peripheral inflammation but not those with axial manifestations (Clegg et al. 1996). A meta-analysis, based on 11 trials, concluded that this agent has a significant impact only on the ESR and the severity of spinal stiffness (Chen and Liu 2006). The primary indication for this agent is a patient who has concomitant peripheral arthritis and has had an inadequate response to NSAIDs and physical modalities. While methotrexate is often used for AS, a meta-analysis concluded that there was no evidence of efficacy and that higher-quality trials and higher dosages of methotrexate were necessary before any definitive conclusions could be drawn (Chen and Liu C 2003). Intra-articular corticosteroids may be helpful when administered into peripheral joints or into the SI joints, if given under fluoroscopic guidance. Systemic steroids are of unproven benefit.

A major advance in the treatment of AS is the development of anti-TNF therapies which block the effect of this cytokine in inflammation. The expression of TNF is increased in SI joint biopsies of AS patients, and overexpression of

TNF leads to sacroiliitis in animal models. Four anti-TNF agents are of proven benefit in AS: infliximab, etanercept, adalimumab, and golimumab (van der Heijde et al. 2005, 2006; Davis et al. 2003; Inman et al. 2008). Infliximab is an IgG1 chimeric monoclonal antibody with the Fab portion derived from the mouse. It is given in a dose of 3–5 mg/kg every 6–8 weeks after initial treatment at 0, 2, and 6 weeks. Etanercept is a recombinant 75-kD TNF receptor IgG1 fusion protein that is self-administered by subcutaneous injection either once (50 mg) or twice (25 mg) weekly. Adalimumab and golimumab are human monoclonal antibodies that are self-administered by subcutaneous injection on alternate weeks (40 mg) or monthly (50 mg), respectively. All of these agents show response rates in 55–60 % of patients for both axial and peripheral inflammation, and responses may be even better in patients with early disease. However, almost all patients relapse when treatment is discontinued. Besides improvement in symptoms and signs of AS, there is improvement in acute-phase reactants, synovial histopathology, and MRI features of inflammation. No evidence indicates that these agents are disease controlling with respect to the prevention of structural damage on plain radiography. Response to treatment appears to be increased in those with short disease duration and high disease activity as indicated by elevated CRP, and worse in those with a long disease duration, impaired function, and no discernible evidence of inflammation on MRI. Even patients with complete spinal ankylosis may benefit from treatment. All these agents are effective for psoriasis, and the monoclonal anti-TNF antibodies, infliximab and adalimumab, also have demonstrated efficacy in both uveitis and colitis. Primary side effects are an increased risk of infection and infusion or injection reactions.

Surgery may be necessary for patients with hip involvement although the results of hip replacement are excellent. Vertebral osteotomy may be required in selected cases to correct marked flexion deformity when forward vision is severely impaired.

Conclusion

Spondyloarthritis is a common disease that manifests early in life and has a strong association with genetic factors such as *HLA-B27*, ERAP-1, and IL23R although their role in the pathogenesis of disease remains unclear. Increasing evidence points to an important role for abnormalities in innate immunity as a driving factor for inflammation. Two major advances have been the introduction of MRI for early diagnosis of sacroiliac joint inflammation and the use of anti-TNF agents for treatment.

References

Braun J, Bollow M, Neure L, Seipelt E, Seyrekbasan F, Herbst H, et al. Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. Arthritis Rheum. 1995;38:499–505.

Burton PR, Clayton DG, Cardon LR, et al. Wellcome trust case control consortium; Australo-Anglo-American spondylitis consortium (TASC). association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet. 2007;39:1329–37.

Chen J, Liu C. Methotrexate for ankylosing spondylitis. Cochrane Database Syst Rev. 2003;3: art. no. CD004524, pub2.

Chen J, Liu C. Is sulfasalazine effective in ankylosing spondylitis? A systematic review of randomized controlled trials. J Rheumatol. 2006;33:722–31.

Clegg DO, Reda DJ, Weisman MH, et al. Comparison of sulfasalazine and placebo in the treatment of ankylosing spondylitis. Arthritis Rheum. 1996;39:2004–12.

Dagfinrud H, Kvien TK, Hagen KB. Physiotherapy interventions for ankylosing spondylitis. Cochrane Database Syst Rev. 2004;4: art. no.CD002822.pub2.DOI.

Davis Jr JC, van der Heijde D, Braun J, et al. Recombinant human tumor necrosis factor receptor (etanercept) for treating ankylosing spondylitis. Arthritis Rheum. 2003;48:3230–3236.

Hammer RE, Maika SD, Richardson JA, Tang JP, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. Cell. 1990;63:1099–112.

Inman RD, Davis Jr JC, Heijde D, et al. Efficacy and safety of golimumab in patients with ankylosing spondylitis: results of a randomized, double-blind, placebo-controlled phase III trial. Arthritis Rheum. 2008;58:3402–12.

Maksymowych WP. Treatment. In: Weisman MH, Reveille JD, van der Heijde D, editors. Ankylosing spondylitis and the spondyloarthropathies. Mosby: St. Louis; 2006. p. 154–86.

Maksymowych WP, Chiowchanwisawakit P, Clare T, et al. Inflammatory lesions of the spine on MRI predict the development of new syndesmophytes in ankylosing spondylitis: evidence of a relationship between inflammation and new bone formation. Arthritis Rheum. 2009;60:93–102.

Reveille JD, Sims AM, Danoy P, et al. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet. 2010;42:123–7.

Reveille JD, Witter JP, Weisman MH. Prevalence of axial spondyloarthritis in the United States: estimates from a cross-sectional survey. Arthritis Care Res. 2012;64:905–10.

Rudwaleit M, van der Heijde D, Landewe R, Listing J, Akkoc N, Brandt J, Braun J, Chou CT, Collantes-Estevez E, Dougados M, Huang F, Gu J, Khan MA, Kirazli Y, Maksymowych WP, Mielants H, Sorensen IJ, Ozgocmen S, Roussou E, Valle-Onate R, Weber U, Wei J, Sieper J. The development of assessment of spondyloarthritis international society (ASAS) classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis. 2009;68:777–83.

Rudwaleit M, van der Heijde D, Landewé R, Akkoc N, Brandt J, Chou CT, Dougados M, Huang F, Gu J, Kirazli Y, Van den Bosch F, Olivieri I, Roussou E, Scarpato S, Sørensen IJ, Valle-Oñate R, Weber U, Wei J, Sieper J. The assessment of spondyloarthritis international society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Ann Rheum Dis. 2011;70:25–31.

Szabo SM, Levy AR, Rao SR, Kirbach SE, Lacaille D, Cifaldi M, Maksymowych WP. Increased risk of cardiovascular and cerebrovascular diseases in individuals with ankylosing spondylitis: a population-based study. Arthritis Rheum. 2011;63:3294–304.

Taurog JD, Dorris ML, Satumtira N, et al. Spondyloarthritis in HLA-B27/human beta2-microglobulin-trangenic rates in not prevented by lack of CD8. Arthritis Rheum. 2009;60:1977–84.

Van der Heijde D, Dijkmans B, Geusens P, et al. Efficacy and safety of infliximab in patients with ankylosing spondylitis: results of a randomized placebo-controlled trial (ASSERT). Arthritis Rheum. 2005;52:582–91.

Van der Heijde D, Kivitz A, Schiff MH, et al. Efficacy and safety of adalimumab in patients with ankylosing spondylitis: results of a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 2006;54:2136–46.

Van der Linden SM, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. Arthritis Rheum. 1984;27:361–8.

Weber U, Østergaard M, Lambert RGW, Maksymowych WP. The impact of MRI on the clinical management of inflammatory arthritides. Skeletal Radiol. 2011;40:1153–73.

Zochling J, van der Heijde D, Dougados M, et al. Current evidence for the management of ankylosing spondylitis: a systematic literature review for the ASAS/EULAR management recommendations in ankylosing spondylitis. Ann Rheum Dis. 2006;65:423–32.

Spondyloarthritis: Psoriatic Arthritis

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Definition

Psoriatic arthritis (PsA) is an inflammatory musculoskeletal disease associated with psoriasis. Although initially described as seronegative for rheumatoid factor, it has become apparent that some patients with PsA do have a positive rheumatoid factor which may be related to age or other reasons. There are specific features of PsA which help distinguish it from other forms of inflammatory arthritis. Because of its association with extra-articular features, peripheral and axial arthritis, radiological sacroiliitis, and HLA*B27, PsA is classified among the seronegative spondyloarthritis (SpA) group of conditions (Wright and Moll 1976 book).

While the first descriptions of PsA were published in the nineteenth century, it was the efforts of Moll and Wright which led to the recognition of PsA as a specific entity. However, while Moll and Wright considered PsA as a benign condition relative to rheumatoid arthritis (RA), over the past several decades, it has become clear that the disease was more frequent and more severe than previously thought, with disease progression, disability, and increased mortality (Gladman 2008).

Pathogenesis

The cause of PsA is unknown. While it occurs primarily among patients with psoriasis, the exact mechanism by which a patient with psoriasis might develop arthritis is unclear. Nonetheless, three factors are considered important: genetic, immunological, and environmental (Haroon and FitzGerald 2012).

Genetic Factors

Both psoriasis and its associated arthritis run in families. Family studies have demonstrated a recurrence risk ratio of 30 for PsA and 4–10 for psoriasis. Several loci have been identified for psoriasis using traditional linkage studies. These include psoriasis susceptibility locus (PSORS) 1 on 6p21.3 [with a number of candidate genes including *HLA-C*06*, *CDSN*, *HCR*, *HERV-K*, *HCG22*, *PSORS1C3*, *POU5F1*, *TCF19*, *CCHCR1*, *LMP*, *SEEK1*, *SPR1*], PSORS2 on 17q [with candidate genes *RUNX1*, *RAPTOR*, *SLC9A3R1*, *NAT9*, *TBCD*, *CARD14*], PSORS3 on 4q [candidate gene *IRF2*], PSORS4 on 1q21.3 [candidate genes Loricrin, Filaggrin, Pglyrp, S100 genes within epidermal differentiation complex], PSORS5 on 3q21 [candidate genes *SLC12A8*, cystatin A, Zn finger protein 148], PSORS6 on 19p [candidate gene *JunB*], PSORS7 on 1p [candidate genes *PTPN22*, *IL23R*], PSORS8 on 16q [candidate genes *CX3CL1*, *CX3R1*, *NOD2/CARD15*], PSORS9 on 4q28–32 [candidate gene *IL15*], and PSORS10 on 18p11 (Chandran 2012). The strongest association is with a locus within the major histocompatibility complex (MHC) on chromosome 6p21 (PSORS1) (Chandran 2012). Fine-mapping studies suggest that HLA-C*0602 is the psoriasis susceptibility gene in this region, although additional susceptibility loci (e.g., MICA) need further evaluation. In PsA there is a stronger association with HLA-B alleles than with HLA-C alleles, while psoriasis (particularly early-onset psoriasis) is associated with HLA-C. The susceptibility locus for PsA may lie more centromeric to that of psoriasis, closer to HLA-B and TNFA.

Several genome-wide association studies (GWAS) in psoriasis have confirmed associations of *HLA*, *IL12B*, *IL23R*, *TNFAIP3*, *TNIP1*, *IL4*, *IL13*, *IL28RA*, *REL*, *IFIH1*, *ERAP1*, *TRAF3IP2*, *NFKBIA*, *TYK2*, *NOS2*, *FBXL19*, *PSMA6-NFKBIA*, *and RNF114* with psoriasis. Several of the GWAS in psoriasis included patients with PsA, thus allowing for comparison between PsA and psoriasis. *HLA-C* and *IL23R* are more strongly associated with psoriasis alone, and *IL12B* with PsA. Several HLA alleles have been found to be associated with PsA. HLA-B*27, HLA-B*08, and HLA-B*38 are significantly more frequent among patients with PsA compared to those with psoriasis alone, while HLA-C*06 is more common among patients with psoriasis alone than those with PsA, although it is increased among PsA patients compared to healthy controls. It has been demonstrated that patients with psoriasis who carry the HLA-C*06 allele develop PsA at a later time in the course of their disease than those who do not carry the allele (Eder et al. 2012).

Thus, a number of susceptibility loci for psoriasis and PsA have been discovered. Although these loci explain only a fraction of the heritability estimates, a model of important pathways in psoriasis pathogenesis is emerging that combines skin barrier function (*LCE3B*, *LCE3C*), TH17 pathway (*IL12B*, *IL23A*, *IL23R*, *TRAF3IP2*, *TYK2*), innate immunity [NFκB and IFN signaling pathway (*TNFAIP3*, *TNIP1*, *NFKBIA*, *REL*, *TYK2*, *IFIH1*, *IL23RA*) and β-defensin], TH2 pathway (*IL4*, *IL13*), and adaptive immunity involving CD8 T cells (ERAP1, ZAP70).

Immunological Factors

The association with class I HLA alleles, the presence of activated state of CD8+ T cells and natural killer (NK) cells in the psoriatic synovium, and the response of the disease to immunomodulatory therapy indicate that the immune system, especially the lymphocytes, plays an important role in PsA pathogenesis. McGonagle and colleagues have proposed that the synovial membrane and entheses form a "synovioentheseal complex." Tissue-specific factors, including microtrauma, lead to regional innate immune activation and persistent

inflammation. The genetic association of PsA with class I HLA alleles, MICA, and KIR genes indicates that PsA is closer to the "autoinflammatory" end rather than the "autoimmune" end of the spectrum of immune-mediated diseases (Benjamin and McGonagle 2009).

There is also evidence that the monocyte-macrophage system plays a major role in the initiation and perpetuation of joint inflammation. Monocytes differentiate into macrophages, osteoclasts, Langerhans cells, or dendritic cells in response to microenvironmental signals. In entheseal tissues, monocytes are the principal cells that infiltrate fibrocartilage. Monocytes are also present in the synovial lining of psoriatic joints, and they infiltrate the subsynovial lining. There is an increased frequency of circulating osteoclast precursors in peripheral blood and synovial tissues of PsA patients. These precursors, derived from circulating CD14+ monocytes, differentiate into osteoclasts after exposure to monocyte colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANKL) expressed by synovial lining cells in inflamed psoriatic synovium and is responsible for bone erosions (Mensah et al. 2008).

Environmental Factors

Associations of psoriatic arthritis have been reported with a host of environmental exposures, including Rubella vaccination, injury sufficient to require a medical consultation, recurrent oral ulcers, moving between homes, bone fractures requiring hospital admission, lifting heavy loads, infections that require treatment with antibiotics, HIV infection, and corticosteroid use. Smoking is less prevalent in PsA patients compared to patients with psoriasis without PsA. *IL13* gene polymorphisms are associated with PsA, especially in nonsmokers (Eder et al. 2012).

Clinical Features

Psoriatic arthritis is an inflammatory musculoskeletal disease which affects the peripheral joints, axial skeleton, and periarticular structures such as entheses and tendons. Moll and Wright described five "predominant" patterns of PsA, including distal, oligoarticular, polyarticular, arthritis mutilans, and spinal disease. However, because these patterns change over time and do not appear to carry prognostic value, more recently PsA is described as peripheral, axial, or combined (Gladman et al. 2005).

Peripheral Arthritis

The majority of patients with PsA present with peripheral arthritis. Early on, the arthritis is likely to be oligoarticular, with four or less joints involved, and asymmetric. However, with time, more joints are involved such that after several years of disease, the majority of patients have polyarticular disease. All joints in the body may be affected, but the most commonly affected at presentation to clinic are the small joints of the hands and feet, followed by wrists, ankles, knees, shoulders, elbows, hips, and the temporomandibular and sternoclavicular joints. The majority of the patients present with arthritis which is inflammatory in nature, presenting with painful swollen joints, which are worse with inactivity and improve with exercise and associated with morning stiffness in at least 50 % of the patients. However, it should be noted that patients with PsA are not as tender as patients with rheumatoid arthritis, and their arthritis is often missed until they develop obvious deformities. A unique feature of PsA is a purplish discoloration over the affected joint. The peripheral arthritis of PsA may be quite destructive and may result in **arthritis mutilans**, where there is complete lysis of a joint leading to a flail joint. At the same time, the arthritis may lead to ankylosis, leading to fused joints. Both of these features are quite devastating to the patient who may become quite disabled.

Dactylitis

Defined as inflammation of a whole digit, this feature is unique to PsA and affects about 48 % of the patients. It affects fingers and toes occasionally in a symmetric distribution. It results from inflammation in joints, bones, tendons, and soft tissues. Acutely it is associated with pain, swelling, and redness but may become chronic when there is no longer pain but persistent

swelling of the digit. Joints of digits with dactylitis are more likely to develop radiological erosions, suggesting that this feature is prognostically important.

Axial Disease

About 50 % of patients with PsA develop axial involvement manifesting with sacroiliitis as well as syndesmophyte formation. However, only 2–4 % of the patients have isolated axial disease. Axial PsA differs from ankylosing spondylitis in its severity and, like the peripheral arthritis, is less painful. It is associated with HLA-B*27 although to a lesser degree than ankylosing spondylitis. Axial disease is associated with more severe peripheral arthritis as well as arthritis mutilans.

Enthesitis

Inflammation at the insertion of tendons and ligaments into bone is a feature of all types of spondyloarthritis, and in PsA it is clinically detectable in approximately 40 % of patients. The most common sites involved include the Achilles tendon and plantar fascia insertions into the calcaneus. However, other sites may be affected as well.

Extra-Articular Manifestations

Skin: By definition most patients with PsA have psoriasis. It seems that patients with severe psoriasis are more likely to develop PsA, although a direct relationship between skin and joint disease has not been demonstrated. Most patients have psoriasis vulgaris or chronic plaque psoriasis, and a minority may have pustular or guttate psoriasis.

Nail disease: Nail lesions are the only clinical manifestation which identifies patients with psoriasis more likely to develop PsA. They occur in more than 85 % of patients with PsA compared to 40–50 % of patients with cutaneous psoriasis alone. Pits and onycholysis are the major features of nail disease in PsA.

Mucous membrane lesions occurring in patients with PsA tend to be painless. However, it should also be noted that mucous membrane lesions may complicate methotrexate therapy, although these tend to be painful. Urethritis occurs in about 7 % of patients with PsA. Inflammatory bowel disease may occur in a proportion of patients with PsA, usually Crohn's disease. Aortic root dilation, which is much less common in PsA than in ankylosing spondylitis, presents with aortic regurgitation and has a characteristic tambour quality of the second heart sound.

Diagnosis

The diagnosis of PsA is based on clinical features and is usually made at the bedside. The type and distribution of joint involvement is helpful, the presence of the purplish discoloration is suggestive, and the combination of peripheral and axial disease is supportive of the diagnosis of PsA. Confirmation may be obtained through laboratory tests demonstrating a negative rheumatoid factor and radiographs showing fluffy periostitis, the presence of the classic pencil-in-cup change as well as ankylosis. A number of classification criteria have been proposed, but the CASPAR criteria are the most widely accepted and used (Table 1).

Since it has become clear that early diagnosis is helpful to provide proper care for patients with PsA and since patients usually present with skin disease prior to the onset of joint disease, it is important for dermatologists to identify patients with psoriasis who are developing PsA. A number of screening tools have been developed and should provide help in identifying patients that should be reviewed by rheumatologists (Dominguez et al. 2010).

Course and Prognosis

Although Moll and Wright described arthritis mutilans as a severe destructive form of the disease, they identified it in only 5 % of their cases. They and others have considered PsA as a mild disease compared to rheumatoid arthritis. However, studies over the past three decades have demonstrated that PsA can be a progressively destructive form of arthritis,

Spondyloarthritis: Psoriatic Arthritis, Table 1 CASPAR criteria for the classification of psoriatic arthritis

Primary requirement: inflammatory musculoskeletal disease (joint, spine, or entheseal) with three or more of the following:		
1. Evidence of psoriasis (one of a, b, c)	(a) Current psoriasis[a]	Psoriatic skin or scalp disease present today as judged by a dermatologist or rheumatologist
	(b) A history of psoriasis	A history of psoriasis that may be obtained from patient, family doctor, dermatologist, or rheumatologist
	(c) Family history of psoriasis	A history of psoriasis in a first- or second-degree relative according to patient report
2. Psoriatic nail dystrophy		Typical psoriatic nail dystrophy including onycholysis, pitting, and hyperkeratosis observed on current physical examination
3. A negative test for rheumatoid factor		By any method except latex but preferably by ELISA or nephelometry, according to the local laboratory reference range
4. Dactylitis either a or b		Swelling of an entire digit
		Recorded by a rheumatologist
5. Radiological evidence of juxta-articular new bone formation		Ill-defined ossification near joint margins (but excluding osteophyte formation) on plain x-rays of hand or foot

Taylor et al. 2006
[a]Current psoriasis scores 2, others 1

associated with marked limitation of activities of daily living, reduced quality of life, and fatigue. Moreover, there is an increased mortality risk. Both progression of joint damage and mortality are predicted by the degree of inflammatory joint disease (Bond et al. 2007; Cresswell et al. 2011). It has also been demonstrated that patients seen within 2 years of diagnosis fare better than those who present later in the course of their disease (Gladman et al. 2011). Thus, patients with PsA should be diagnosed and treated early in order to prevent these untoward outcomes.

Treatment

Treatment of PsA includes treatment of skin and joint manifestations. Ideally, approaches which address both aspects of the disease should be used. There are patients with PsA whose major issue is the joint disease and in whom the skin disease is minimal. If the joint disease is mild, nonsteroidal anti-inflammatory medications may be sufficient. However, if the arthritis persists, then disease-modifying antirheumatic drugs (DMARDs) should be used. Unfortunately there is limited evidence for efficacy for most DMARDs. Methotrexate has been the most commonly used DMARD in PsA, and it is helpful for psoriatic skin lesions. However, clinical trials have not demonstrated efficacy for joint disease. Nonetheless, most therapeutic guidelines for the treatment of PsA recommend using methotrexate as the first-line DMARD (Ash et al. 2012).

Leflunomide has also been used with some success. Neither drug has demonstrated an effect on progression of radiographic damage. Other DMARDs used in PsA include sulfasalazine, which has been shown to have a marginal therapeutic advantage over placebo, and cyclosporine, which works for the clinical signs and symptoms but is more toxic than other drugs.

Based on the pathogenesis of the disease, anti-TNF agents have been used since 2000 with remarkable results. These medications are effective for the signs and symptoms of the disease as well as for prevention of progression of joint damage. There is some evidence that damage may be reversed with the use of these drugs. Thus, the management of PsA has been facilitated in the past decade. Nonetheless, not all patients respond to the treatment currently available and additional medications are required. Other biologic therapies including IL-12/23 inhibitors, IL-17 inhibitors, and IL-6 inhibitors are being investigated and are promising.

The Group for Research and Assessment of Psoriasis and Psoriatic Arthritis provided

international recommendations for the management of PsA. These recommendations reflect the five domains of PsA including peripheral joints, axial disease, skin and nail disease, dactylitis, and enthesitis. The choice of therapy would be dictated by the domain which is most severe in each patient (Ritchlin et al. 2009).

Cross-References

- ▶ Autoinflammatory Diseases
- ▶ Psoriasis
- ▶ Spondyloarthritis: Reactive Arthritis

References

Ash Z, Gaujoux-Viala C, Gossec L, Hensor EM, FitzGerald O, Winthrop K, et al. A systematic literature review of drug therapies for the treatment of psoriatic arthritis: current evidence and meta-analysis informing the EULAR recommendations for the management of psoriatic arthritis. Ann Rheum Dis. 2012;71:319–26.

Benjamin M, McGonagle D. The enthesis organ concept and its relevance to the spondyloarthropathies. Adv Exp Med Biol. 2009;649:57–70.

Bond SJ, Farewell VT, Schentag CT, Gladman DD. Predictors for radiological damage in psoriatic arthritis. Results from a single centre. Ann Rheum Dis. 2007;66:370–6.

Chandran V. The genetics of psoriasis and psoriatic arthritis. Clin Rev Allergy Immunol 2013;44:149–56.

Cresswell L, Chandran V, Farewell VT, Gladman DD. Inflammation in an individual joint predicts damage to that joint in psoriatic arthritis. Ann Rheum Dis. 2011;70:305–8.

Dominguez P, Gladman DD, Helliwell P, Mease PJ, Husni ME, Qureshi AA. Development of screening tools to identify psoriatic arthritis. Curr Rheumatol Rep. 2010;12:295–9.

Eder L, Chandran V, Pellet F, Shanmugarajah S, Rosen CF, Bull SB, Gladman DD. Human leukocyte antigen risk alleles for psoriatic arthritis among psoriasis patients. Ann Rheum Dis. 2012;71:50–5.

Gladman DD. Mortality in psoriatic arthritis. Clin Exp Rheumatol. 2008;26(5):s62–5.

Gladman DD, Antoni C, Clegg D, Mease P, Nash P. Psoriatic arthritis – epidemiology and clinical features. Ann Rheum Dis. 2005;64(II):14–7.

Gladman DD, Thavaneswaran A, Chandran V, Cook RJ. Do patients with psoriatic arthritis who present early fare better than those presenting later in the disease? Ann Rheum Dis. 2011;70:2152–4.

Haroon M, Fitzgerald O. Pathogenetic overview of psoriatic disease. J Rheumatol Suppl. 2012;89:7–10.

Mensah KA, Schwarz EM, Ritchlin CT. Altered bone remodeling in psoriatic arthritis. Curr Rheumatol Rep. 2008;10:311–7.

Ritchlin CT, Kavanaugh A, Gladman DD, Mease PJ, Boehncke WH, de Vlam K, Fiorentino D, FitzGerald O, Gottlieb AB, McHugh N, Nash PT, Qureshi AA, Soriano ER, Taylor WJ. Treatment recommendations for psoriatic arthritis. Ann Rheum Dis. 2009;68:1387–94.

Taylor WJ, Gladman DD, Helliwell PS, Marchesoni A, Mease P, Mielants H. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum. 2006;54:2665–73.

Wright V, Moll JMH. Psoriatic arthritis. In: Wright V, Moll JMH, editors. Seronegative arthritis. Amsterdam: North Holland; 1976. p. 169–235.

Spondyloarthritis: Reactive Arthritis

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Synonyms

Arthritis urethritica; Fiessinger-Leroy syndrome; Polyarteritis enterica; Reactive arthritis; Reiter syndrome; Sexually acquired reactive arthritis (SARA)

Definition

Reactive arthritis (ReA) is an inflammatory arthritis that occurs within 1–6 weeks after the patient is exposed to one of the triggering bacterial organisms. ReA belongs to the group of arthritides known as the spondyloarthropathies. Two main types of ReA have been described, post-venereal and post-dysentery (or post-enteric) ReA.

Historical Background

Reactive arthritis (ReA) is an inflammatory joint disease that belongs to the group of arthritides

known as the spondyloarthropathies. These are a group of arthritides that share clinical, radiographic, and laboratory features. The history of ReA is confusing because multiple terms and eponyms have been used in the literature throughout the years to describe this condition. The most commonly used historical term to describe this condition was Reiter syndrome, but Fiessinger-Leroy syndrome, sexually acquired reactive arthritis (SARA), arthritis urethritica, and polyarteritis enterica also have been utilized to describe this same condition.

The eponym Reiter syndrome is a term that should no longer be utilized for several reasons. First, a general push in the medical literature exists to avoid the use of eponyms. Second, this eponym is used to denote the clinical triad of conjunctivitis, urethritis, and arthritis; this triad is far too restrictive to describe ReA, and it is even possible for ReA to present with the complete absence of these three "classic" symptoms. Thus, a more descriptive term is preferred. Third, contrary to common belief, Hans Conrad Reiter was not the first to describe the condition; there are many well-documented cases of ReA in the medical literature long before Reiter's description (Carter and Hudson 2009). Finally, there is a moral issue with the use of the eponym because during World War II, Hans Reiter performed unauthorized medical experiments on concentration camp victims. After World War II, Reiter was charged with "overtly criminal" acts that included involuntary sterilization, involuntary vaccination with typhus, and euthanasia (Keynan and Rimar 2008). For these reasons, the term ReA is the current and most appropriate term for this disease process.

Spondyloarthritis: Reactive Arthritis, Table 1 Triggering microbes of reactive arthritis (Reprinted from Carter (2006) with permission from Elsevier)

Definite causes
Post-venereal:
Chlamydia trachomatis
Post-enteric:
Salmonella (S. enteritidis, S. typhimurium, S. bovismorbificans, S. blockley)
Shigella (S. flexneri, S. dysenteriae, S. sonnei, S. boydii)
Campylobacter (C. jejuni, C. coli)
Yersinia (Y. enterocolitica, Y. pseudotuberculosis)
Probable causes
Chlamydophila (Chlamydia) pneumoniae
Ureaplasma urealyticum
Bacille Calmette-Guerin (intravesicular)
Possible causes
Bacillus cereus
Brucella abortus
Clostridium difficile
Escherichia coli
Helicobacter pylori
Hafnia alvei
Lactobacillus
Neisseria meningitidis serogroup B
Pseudomona
Intestinal parasites (*Strongyloides stercoralis, Taenia saginata, Giardia lamblia, Ascaris lumbricoides, Filariasis*, and *Cryptosporidium)*
Other types of inflammatory arthritis in which bacteria may play a causative role
Borrelia burgdorferi (Lyme disease)
Propionibacterium acnes (SAPHO)
Streptococcus sp (post-streptococcal reactive arthritis)
Trophyrema whippelii (Whipple's disease)

Disease Description

ReA is an inflammatory arthritis that occurs within 1–6 weeks after the patient is exposed to one of the triggering bacterial organisms. Two main types of ReA have been described, post-venereal and post-dysentery (or post-enteric) ReA. Although many triggering organisms have been implicated for both variants (Table 1), definitive triggers of post-dysentery ReA include *Salmonella, Shigella, Campylobacter*, and *Yersinia*, while *Chlamydia trachomatis* is a definitive trigger of the post-venereal variant. Other possible etiologic triggers include *Chlamydia pneumoniae*, *Ureaplasma urealyticum*, *H. pylori*, and various intestinal parasites. Reports of ReA secondary to *E. coli* (Townes et al. 2008), *C. difficile* (Birnbaum et al. 2008), and intravesicular Bacillus Calmette-Guerin (BCG) (Tinazzi et al. 2006) have garnered recent recognition.

Because certain bacteria are known to be etiologic of this condition, ReA represents the classic interplay between host and environment. Many studies have been performed in hopes of unraveling the pathogenesis of this condition. In general, these organisms are acquired through the gastrointestinal or genitourinary tract. Remarkably, many different studies performed in several different laboratories have demonstrated that these bacteria traffic from the initial site of infection to the synovial tissue (Gerard et al. 1998). These synovial-based bacteria are not detectable by routine culture, so it was previously felt that ReA was a sterile arthritis resulting from a bacterial trigger. However, the routine documentation of these organisms in synovial samples by polymerase chain reaction has called into question this disease description. It is generally accepted that these bacteria travel through the peripheral blood in macrophages and monocytes until they ultimately reside in the synovial tissue and/or fluid. Even more intriguing, synovial samples from patients with *Chlamydia*-induced ReA have demonstrated these synovial-based chlamydiae to be viable (Gerard et al. 1998). They exist in an aberrant, persistent form, but these chlamydiae are intact and metabolically active. Similar studies have been performed on patients with the post-dysentery variant of ReA; with one exception (Gaston et al. 1999), these data demonstrate that these causative organisms also traffic to the synovial compartment, but only bacterial fragments have been demonstrated (Nikkari et al. 1999). The synovial-based post-dysentery organisms are no longer viable.

While the true significance of viable versus nonviable synovial-based organisms is not yet fully defined, these findings could have important treatment implications, specifically regarding the use of antibiotics (discussed in the Treatment section). The mystique that surrounds this pathophysiology is further heightened by fact that the phenotypic disease characteristics of post-dysentery and post-venereal ReA are generally congruent. These same bacteria have rarely been demonstrated in synovial samples of patients with osteoarthritis and even normal controls (Schumacher et al. 1999). However, data demonstrate that these etiologic synovial-based bacteria are significantly more prevalent in patients with ReA compared to osteoarthritis (Carter et al. 2009).

Epidemiology

The lack of a standard disease definition, well-validated diagnostic criteria, and the uses of multiple terms and eponyms to describe this condition makes epidemiologic studies problematic. Because only a minority of patients exposed to the causative organisms will develop ReA, the epidemiology of ReA includes not only the incidence and prevalence of the disease but also the attack rate (i.e., the percentage of individuals who develop the condition after acquiring one of the etiologic infections). The incidence, prevalence, and attack rate of ReA vary widely among different studies. The variability of the genetic background of the population studied, differences in local environmental factors, and differing arthritogenic propensity of the several etiologic organisms offer some explanation for the variability of the epidemiologic data in the literature. Interestingly, the arthritogenic propensity of these organisms appears to vary even between different species within the same genus. For example, recent data suggest that within the *Chamydia* genus, the ocular serovars, not the genital species, appear to be particularly arthritogenic; they may be uniquely arthritogenic within the genus (Gerard et al. 2010). Other observational data demonstrate similar findings with the post-dysentery organisms. For example, in 2008 there was a large outbreak of *Salmonella saint-paul* in the USA and Canada without any known subsequent reported cases of ReA. Yet other species of *Salmonella* (e.g., *S. typhimurium*, *S. enteritidis*) are well-known causes of ReA.

The post-dysentery form of ReA affects males and females with the same frequency, whereas the post-venereal form occurs more commonly in males. It has been stated that the male to female ratio of post-venereal ReA is 9:1; it is difficult to know the true ratio because women often present

with more subtle clinical symptoms, and the triggering *Chlamydia trachomatis* infection is more likely to be asymptomatic in women than men. Adults are more likely to develop ReA than children. The attack rate of post-dysentery ReA generally ranges from 1.5 % (Eastmond et al. 1983) to about 30 % (Dworkin et al. 2001) depending on the study and causative organism; however, one recent study of self-reported cases suggested as many as 63 % of patients experienced symptoms consistent with ReA after an acute *Salmonella* infection (Rohekar et al. 2008). ReA is thought to occur in about 5 % of individuals who develop an acute *Chlamydia trachomatis* infection (Rich et al. 1996).

It is also possible – perhaps even likely – that ReA is underdiagnosed. A review of the prevalence of the triggering organisms and expected attack rates suggest that the incidence of ReA exceeds that of rheumatoid arthritis (Carter and Hudson 2009). A final complicating factor involving the epidemiology of ReA is that the condition spontaneously remits as up to 70 % of cases.

Clinical, Radiographic, and Laboratory Features

As stated, ReA is a type of spondyloarthritis. The spondyloarthritidopathies include ankylosing spondylitis, inflammatory bowel disease-related arthritis, psoriatic arthritis, ReA, and undifferentiated spondyloarthritis. The acute and chronic symptoms (see Table 2) can include articular, tendon, mucosal, cutaneous, ocular, and occasionally cardiac manifestations or systemic features (fever, malaise, weight loss); the latter are usually confined to the acute stage. Symptoms typically start within 1–6 weeks of the initial infection.

The inflammatory arthritis of ReA can be an axial or peripheral arthritis, or combinations of both. The axial inflammatory arthritis typically involves the sacroiliac joints and lumbar spine and can spread caudally over time. The peripheral arthritis has a predilection for the large joints of the lower extremities. More data are needed to better define the percentage of patients who present with axial symptoms, peripheral symptoms, or both.

Over time, this inflammatory arthritis can lead to radiographic damage. The radiographic features of ReA include sacroiliitis, periostitis and periosteal new bone formation, non-marginal syndesmophytes, joint erosions, and joint space narrowing. Sacroiliac involvement tends to be asymmetric and typical spinal features include non-marginal syndesmophytes. The peripheral arthritis can also lead to permanent radiographic damage. In general, the radiographic features of ReA mimic those of psoriatic arthritis. Growing evidence demonstrate that MRI is quite useful at detecting the acute inflammatory changes of sacroiliitis (i.e., pre-radiographic sacroiliitis).

Common extrarticular features include enthesitis, cutaneous/mucosal, and ocular manifestations. Enthesitis is inflammation at the transitional zone where collagenous structures such as tendons and ligaments insert into bone. These sites are unique in that the tendons insert directly into trabecular bone. This is a hallmark feature of any of the spondyloarthropathies, including ReA. Common types of enthesitis in ReA are Achilles tendonitis and plantar fasciitis, but inflammation can occur at any enthesis. Typical cutaneous features include a pustular and/or psoriasiform rash of the palms, soles, or penis; shallow oral ulcers are also commonly seen. Ocular involvement includes both conjunctivitis and/or anterior uveitis (iritis).

Regarding laboratory features, it is important to remember that ReA can follow at least two disease courses. The first is an acute syndrome occurring shortly after the triggering infection followed by gradual resolution of the symptoms, the second begins in a similar fashion yet can progress to chronic disease. During the acute stage, individuals will often have elevated acute phase reactants such as an elevated erythrocyte sedimentation rate (ESR) or C-reactive protein. Conversely, patients with chronic ReA typically display normal inflammatory markers. Patients in the acute phase might also display other indicators of inflammatory response including leukocytosis and/or thrombocytosis.

Spondyloarthritis: Reactive Arthritis, Table 2 Clinical manifestations of reactive arthritis (Reprinted from Carter (2006) with permission from Elsevier)

Acute symptoms		
Articular		
Most commonly present with oligoarthritis, but can also present with polyarthritis or monoarthritis		
Axial:	*Frequently involved:*	
	Sacroiliac joints	
	Lumbar spine	
	Occasionally involved:	
	Thoracic spine (usually seen in chronic ReA)	
	Cervical spine (usually seen in chronic ReA)	
	Cartilagenous joints (symphysis pubis; sternoclavicular and costosternal joints)	
Peripheral:	*Frequently involved:*	
	Large joints of the lower extremities (especially knees)	
	Dactylitis (sausage digit): Very specific for a spondyloarthropathy	
Enthesitis		
Hallmark feature		
Inflammation at the transitional zone where collagenous structures such as tendons and ligaments insert into bone		
Common sites: Plantar fasciitis, Achilles tendonitis; but any enthesis can be involved		
Mucosal		
Oral ulcers (generally painless)		
Sterile dysuria (occurs with both post-venereal and post-dysentery forms)		
Cutaneous		
Keratoderma blenorrhagicum:	Pustular or plaque-like rash on the soles and/or palms	
	Grossly and histologically indistinguishable from pustular psoriasis	
	Can also involve nails (onycholysis, subungual keratosis, nail pits), scalp, extremities	
Circinate Balanitis:	Erythema or plaque-like lesions on the shaft and/or glans of penis	
Ocular		
Conjunctivitis:	Typically during acute stages only	
Anterior Uveitis (iritis):	Often recurrent	
Rarely described:	Scleritis, pars planitis, iridocyclitis, and others	
Cardiac		
Pericarditis (uncommon)		
Chronic symptoms (>6 months)		
Articular		
Axial:	Sacroiliac joints	
	Lumbar spine	
	Thoracic spine	
	Cervical spine	
	Cartilagenous joints (symphysis pubis; sternoclavicular joints)	
Peripheral:	Large joints of the lower extremities (especially knees)	
Dactylitis (sausage digit):	Very specific for a spondyloarthropathy	
Enthesitis		
Chronic inflammation can cause collagen fibers to undergo metaplasia forming fibrous bone		
Chronic enthesitis leads to radiographic findings:		
Plantar/Achilles spurs		
Periostitis		
Non-marginal syndesmophytes		
Syndesmoses of the sacroiliac joints		

(*continued*)

Spondyloarthritis: Reactive Arthritis, Table 2 (continued)

Mucosal	
Sterile dysuria	
Cutaneous	
Keratoderma blennorrhagicum	
Circinate Balanitis	
Ocular	
Anterior Uveitis (iritis):	Often recurrent
Rarely described:	Scleritis, pars planitis, iridocyclitis, and others
Cardiac	
Aortic regurgitation	
Valvular pathologies	

HLA-B27 is felt to increase susceptibility to ReA, but the data suggest there may be too much emphasis on this HLA haplotype. The majority of the data suggest an HLA-B27 prevalence of 30–50 % in patients who develop ReA (Carter and Hudson 2009). Recent reports indicate that HLA-B27 plays little role in determining post-enteric ReA susceptibility (Carter and Hudson 2009); the same is likely to be true for post-chlamydial ReA. Rather than truly increasing disease susceptibility, HLA-B27 positive patients may have more severe symptoms thereby making the condition more clinically apparent (Schiellerup et al. 2008).

Treatment

Although there are no well-designed prospective trials assessing the efficacy of NSAIDs in the treatment of ReA, these remain the mainstay of therapy for the inflammatory arthritis of this condition. For more severe cases, corticosteroids can be utilized; data suggest these are more effective for the peripheral arthritis and less so for the axial symptoms (Flores et al. 2003). Topical corticosteroids are also quite useful for treating the ocular and/or cutaneous manifestations of ReA.

Disease modifying antirheumatic drugs (DMARDs) have been utilized in the treatment of ReA; these medications probably have a better role for treating those with chronic disease. The best studied DMARD in the setting of ReA is sulfasalazine. In a large Veterans Administration study, sulfasalazine demonstrated an overall trend of efficacy, but not statistically significant improvement compared to placebo (Clegg et al. 1996). Methotrexate, azathioprine, and cyclosporine have been advocated as potential treatments for ReA but never formally evaluated in a prospective trial.

The tumor necrosis factor (TNF)-alpha antagonists have demonstrated great success in the treatment of other types of spondyloarthritis, but there are no randomized trials in ReA to assess the efficacy of these biological therapies. Several case reports, a small open-label study, and a retrospective analysis of ten patients suggest clinical benefit with these drugs in the treatment of ReA (Meyer et al. 2011). However, there are theoretical concerns regarding TNF-alpha antagonism in ReA. Chlamydiae are the most common trigger of ReA and in vitro data suggest that growth and infectivity of *Chlamydia trachomatis* are inversely associated with TNF-alpha levels (Ishihara et al. 2005). The general lack of viability of the post-enteric organisms in the setting of ReA suggests that anti-TNF therapy might be more appropriate in these patients.

The fact that ReA is triggered by bacteria and because long-term viability of the organism in the synovium has been demonstrated (at least in the case of *Chlamydia*-induced ReA) suggest a potential role for antibiotics. One of the earliest trials assessing the role of antibiotics in ReA analyzed 3 months of treatment with lymecycline in patients with acute ReA (Lauhio et al. 1991). This study demonstrated no benefit

to patients with post-dysentery ReA, whereas there was improvement in patients with *Chlamydia*-induced ReA. The initial optimism generated by this study was dampened by several follow-up studies suggesting that antibiotics were not effective for ReA. However, these follow-up studies did not separate post-venereal and post-dysentery patients and they included patients with chronic disease. More recent data suggest that 6-months of antibiotics are efficacious if utilized specifically in those ReA patients whose disease was triggered by chlamydiae and if a combination of two antibiotics are used (Carter et al. 2010).

Conclusion

Although the notion of viruses and/or bacteria being etiologic to chronic human disease has been proposed for many years, ReA is one of a few human diseases with a definitive bacterial trigger. This insight into disease initiation has led to significant advances in understanding of the pathophysiology of this condition. However, the more we discover about this classic interplay between host and environment, the more questions arise. Interestingly, the post-venereal and post-dysentery variants are clinically congruent but have surprising differences in their pathophysiology. These same differences could have important treatment implications.

Cross-References

- ▶ Inflammatory Bowel Disease
- ▶ Spondyloarthritis: Ankylosing Spondylitis
- ▶ Spondyloarthritis: Psoriatic Arthritis

References

Birnbaum J, Bartlett JG, Gelber AC. Clostridium difficile: an under-recognized cause of reactive arthritis? Clin Rheumatol. 2008;27(2):253–5.

Carter JD. Reactive arthritis: defined etiologies, emerging pathophysiology, and unresolved treatment. Infect Dis Clin North Am. 2006;20:827–847

Carter JD, Hudson AP. Reactive arthritis: clinical aspects and medical management. Rheum Dis Clin North Am. 2009;35(1):21–44.

Carter JD, Gérard HC, Espinoza LR, Ricca LR, Valeriano J, Snelgrove J, Oszust C, Vasey FB, Hudson AP. Chlamydiae as etiologic agents in chronic undifferentiated spondylarthritis. Arthritis Rheum. 2009;60(5):1311–6.

Carter JD, Espinoza LR, Inman RD, Sneed KB, Ricca LR, Vasey FB, Valeriano J, Stanich JA, Oszust C, Gerard HC, Hudson AP. Combination antibiotics as a treatment for chronic *Chlamydia*-induced reactive arthritis: a double-blind, placebo-controlled, prospective study. Arthritis Rheum. 2010;62(5):1298–307.

Clegg DO, Reda DJ, Weisman MH, et al. Comparison of sulfasalazine and placebo in the treatment of reactive arthritis (Reiter's syndrome). A Department of Veterans Affairs Cooperative Study. Arthritis Rheum. 1996;39(12):2021–7.

Dworkin MS, Shoemaker PC, Goldoft MJ, Kobayashi JM. Reactive arthritis and Reiter's syndrome following an outbreak of gastroenteritis caused by Salmonella enteritidis. Clin Infect Dis. 2001;33(7):1010–4.

Eastmond CJ, Rennie JA, Reid TM. An outbreak of Campylobacter enteritis – a rheumatological follow up survey. J Rheumatol. 1983;10(1):107–8.

Flores D, Marquez J, Garza M, Espinoza LR. Reactive arthritis: newer developments. Rheum Dis Clin North Am. 2003;29(1):37–59.

Gaston JS, Cox C, Granfors K. Clinical and experimental evidence for persistent Yersinia infection in reactive arthritis. Arthritis Rheum. 1999;42(10):2239–42.

Gerard HC, Branigan PJ, Schumacher Jr HR, Hudson AP. Synovial *Chlamydia trachomatis* in patients with reactive arthritis/Reiter's syndrome are viable but show aberrant gene expression. J Rheumatol. 1998;25(4):734–42.

Gerard HC, Stanich JA, Whittum-Hudson JA, Schumacher HR, Carter JD, Hudson AP. Patients with Chlamydia-associated arthritis have ocular (trachoma), not genital, serovars of *C. trachomatis* in synovial tissue. Microb Pathog. 2010;48(2):62–8.

Ishihara T, Aga M, Hino K, Ushio C, Taniguchi M, Iwaki K, Ikeda M, Kurimoto M. Inhibition of *Chlamydia trachomatis* growth by human interferon-alpha: mechanisms and synergistic effect with interferon-gamma and tumor necrosis factor-alpha. Biomed Res. 2005;26(4):179–85.

Keynan Y, Rimar D. Reactive arthritis – the appropriate name. Isr Med Assoc J. 2008;10:256–8.

Lauhio A, Leirisalo-Repo M, Lahdevirta J, Saikku P, Repo H. Double-blind, placebo-controlled study of three-month treatment with lymecycline in reactive arthritis, with special reference to *Chlamydia arthritis*. Arthritis Rheum. 1991;34(1):6–14.

Meyer A, Chatelus E, Wendling D, Berthelot JM, Dernis E, Houvenagel E, Morel J, Richer O, Schaeverbeke T, Gottenberg JE, Sibilia J. Club rhumatisme et inflammation. Safety and efficacy of

anti-tumor necrosis factor α therapy in ten patients with recent-onset refractory reactive arthritis. Arthritis Rheum. 2011;63(5):1274–80.
Nikkari S, Rantakokko K, Ekman P, Möttönen T, Leirisalo-Repo M, Virtala M, Lehtonen L, Jalava J, Kotilainen P, Granfors K, Toivanen P. Salmonella-triggered reactive arthritis: use of polymerase chain reaction, immunocytochemical staining, and gas-chromatography-mass spectrometry in the detection of bacterial components from synovial fluid. Arthritis Rheum. 1999;42(1):84–9.
Rich E, Hook 3rd EW, Alarcon GS, Moreland LW. Reactive arthritis in patients attending and urban sexually transmitted disease clinic. Arthritis Rheum. 1996;39(7):1172–7.
Rohekar S, Tsui FW, Tsui HW, Xi N, Riarh R, Bilotta R, Inman RD. Symptomatic acute reactive arthritis after an outbreak of salmonella. J Rheumatol. 2008;35(8):1599–602.
Schiellerup P, Krogfelt KA, Locht H. A comparison of self-reported joint symptoms following infection with different enteric pathogens: effect of HLA-B27. J Rheumatol. 2008;35(3):480–7.
Schumacher Jr HR, Arayssi T, Crane M, Lee J, Gerard H, Hudson AP, Klippel J. *Chlamydia trachomatis* nucleic acids can be found in the synovium of some asymptomatic subjects. Arthritis Rheum. 1999;42(6):1281–4.
Tinazzi E, Ficarra V, Simeoni S, Artibani W, Lunardi C. Reactive arthritis following BCG immunotherapy for urinary bladder carcinoma: a systematic review. Rheumatol Int. 2006;26(6):481–8.
Townes JM, Deodhar AA, Laine ES, Smith K, Krug HE, Barkhuizen A, Thompson ME, Cieslak PR, Sobel J. Reactive arthritis following culture-confirmed infections with bacterial enteric pathogens in Minnesota and Oregon: a population-based study. Ann Rheum Dis. 2008;67(12):1689–96.

Subepidermal Blistering Diseases: Bullous Pemphigoid

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Synonyms

Benign pemphigus; Old-age pemphigus; Parapemphigus; Pemphigoid; Senile dermatitis herpetiformis

Definition

Bullous pemphigoid (BP), which is often referred to as "pemphigoid," is the most common autoimmune blistering disease of the elderly. Usually a chronic disease, it is characterized by spontaneous exacerbations and potentially appreciable morbidity.

Clinical

Bullous pemphigoid typically presents with a generalized, intensely pruritic eruption. Early erythematous lesions may be eczematous or urticarial and evolve into tense blisters and erosions, rarely with mucous membrane involvement. A broad spectrum of clinical presentation exists, and that polymorphic nature leads to frequent misdiagnoses during the early stages when overt bullae are absent. Lesions may be localized, and there may be appreciable morbidity.

Bullous pemphigoid has become the paradigm for autoimmune disease understanding, with the target antigens being well characterized and their genes cloned.

Pemphigoid gestationis is an intensely pruritic bullous eruption that is associated with pregnancy. Autoantibodies most often target BP180. A transient eruption in newborns may be observed after transplacental transfer of IgG autoantibodies from affected mothers.

Numerous medications and physical vehicles such as wounds, trauma, and UV light have been reported in association with the drug-induced form of bullous pemphigoid, but there are no well-defined causal agents. Bullous pemphigoid has been associated with other inflammatory skin conditions such as psoriasis (▶ PSORIASIS-PATHOGENESIS) and lichen planus as well as with systemic disorders such as diabetes mellitus, rheumatoid arthritis (RA-PATHOGENESIS), and ulcerative colitis (IBD), and neurological diseases such as multiple sclerosis and Parkinson's disease (Ahmed et al. 1982).

On histopathologic examination, these blisters are subepidermal with viable epidermis forming the roof. A sparse or dense infiltrate of

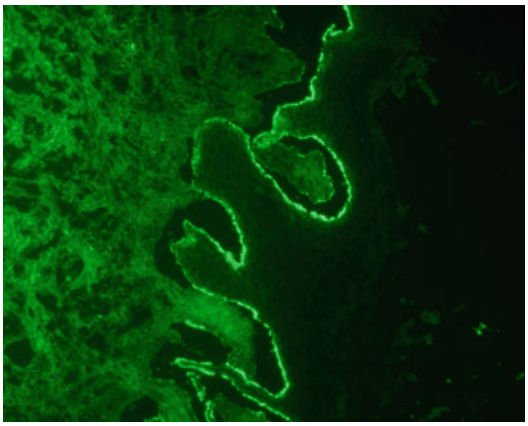
neutrophils, eosinophils, and other inflammatory cells is observed particularly in the upper dermis.

Pathophysiology

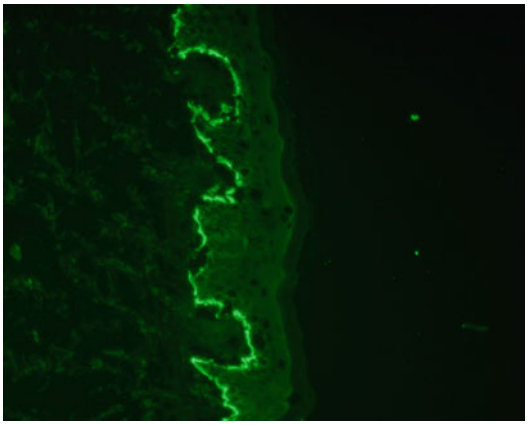
Circulating and tissue-bound pathogenic antibodies (chiefly IgG1 and IgG4 but also IgA) target the 180 kDa bullous pemphigoid antigen (BP180, BPAG-2, type XVII collagen, or LAD1) and the 230 kDa bullous pemphigoid antigen (BP230 or BPAG-1), which are both components of basement membrane zone hemidesmosomes. Hemidesmosomes are junctional complexes that function in dermal-epidermal adhesion. BP230, which is an intracellular cytoplasmic plakin that is localized to the dense plaque, is targeted most commonly at the carboxy-terminal end that mediates the interaction of the hemidesmosome with cellular keratin intermediate filaments (Ishiko et al. 1993; Miller et al. 1993; Skaria et al. 2000). For the transmembrane BP180 molecule, the extracellular NC16A domain is the primary immunodominant epitope. The carboxy-terminal, the intracellular region, and the 97 kDa collagenous ectodomain (LABD97), which is shed normally under physiologic conditions, also serve as targets in some patients (Di Zenzo et al. 2004; Hofmann et al. 2002; Zillikens et al. 1997).

Pathogenesis involves both a humoral and cell-mediated response. Autoantibodies are deposited along the basement membrane zone, with binding C3 along the dermal-epidermal junction. Complement is essential for blister formation in bullous pemphigoid (Schmidt-Ullrich et al. 1975; Anhalt et al. 1981). The activated complement sets off an inflammatory cascade that attracts T cells (both Th1 and Th2), macrophages, mast cells, neutrophils, eosinophils, and other inflammatory mediators such as γ-interferon (γ-IFN), interleukin (IL)-4, IL-5, IL-6, IL-8, and IL-13 (Chen et al. 2002; Liu et al. 1997; Schmidt et al. 2000). Released lysosomal enzymes and proteases, such as matrix metalloproteinase-9 and neutrophil elastase, cleave BP180 and other extracellular matrix proteins and disrupt the hemidesmosome and forming blisters (Verraes et al. 2001; Shimanovich et al. 2004). B cells are further stimulated to produce pathogenic antibodies by autoreactive T cells. Complement, macrophages, T cells, and neutrophils have all been independently shown to be necessary to the process, but the specific sequence and pathways are not fully understood (Chen et al. 2002; Liu et al. 1995, 1997).

Direct IF, which is performed perilesionally as immunoreactants are often lost on the blister roof, shows IgG (Fig. 1) and C3 (or C3 alone) (Fig. 2)



Subepidermal Blistering Diseases: Bullous Pemphigoid, Fig. 1 IgG along the basement membrane zone at the dermal-epidermal junction in bullous pemphigoid, with clefting and early blister formation visible



Subepidermal Blistering Diseases: Bullous Pemphigoid, Fig. 2 C3 deposited along the basement membrane zone at the dermal-epidermal junction in bullous pemphigoid

along the basement membrane zone. Rarely, IgA and IgM deposition may also be observed. Indirect IF is positive in 75 % of patients, false-positive reactions are rare, and the circulating antibodies are diagnostic.

Cross-References

► Psoriasis

References

Ahmed AR, et al. Bullous pemphigoid and ulcerative colitis. Int J Dermatol. 1982;21(10):594–8.

Anhalt GJ, et al. Pathogenic effects of bullous pemphigoid autoantibodies on rabbit corneal epithelium. J Clin Invest. 1981;68(4):1097–101.

Chen R, et al. Macrophages, but not T and B lymphocytes, are critical for subepidermal blister formation in experimental bullous pemphigoid: macrophage-mediated neutrophil infiltration depends on mast cell activation. J Immunol. 2002;169(7):3987–92.

Di Zenzo G, et al. Characterization of the anti-BP180 autoantibody reactivity profile and epitope mapping in bullous pemphigoid patients. J Invest Dermatol. 2004;122(1):103–10.

Hofmann S, et al. Severity and phenotype of bullous pemphigoid relate to autoantibody profile against the NH2- and COOH-terminal regions of the BP180 ectodomain. J Invest Dermatol. 2002;119(5):1065–73.

Ishiko A, et al. Human autoantibodies against the 230-kD bullous pemphigoid antigen (BPAG1) bind only to the intracellular domain of the hemidesmosome, whereas those against the 180-kD bullous pemphigoid antigen (BPAG2) bind along the plasma membrane of the hemidesmosome in normal human and swine skin. J Clin Invest. 1993;91(4):1608–15.

Liu Z, et al. The role of complement in experimental bullous pemphigoid. J Clin Invest. 1995;95(4):1539–44.

Liu Z, et al. A major role for neutrophils in experimental bullous pemphigoid. J Clin Invest. 1997;100(5):1256–63.

Miller JE, Rico MJ, Hall 3rd RP. IgG antibodies from patients with bullous pemphigoid bind to fusion proteins encoded by BPAg1 cDNA. J Invest Dermatol. 1993;101(6):779–82.

Schmidt E, et al. Autoantibodies to BP180 associated with bullous pemphigoid release interleukin-6 and interleukin-8 from cultured human keratinocytes. J Invest Dermatol. 2000;115(5):842–8.

Schmidt-Ullrich B, et al. Ultrastructural localization of in vivo-bound complement in bullous pemphigoid. J Invest Dermatol. 1975;65(2):217–9.

Shimanovich I, et al. Granulocyte-derived elastase and gelatinase B are required for dermal-epidermal separation induced by autoantibodies from patients with epidermolysis bullosa acquisita and bullous pemphigoid. J Pathol. 2004;204(5):519–27.

Skaria M, et al. IgG autoantibodies from bullous pemphigoid patients recognize multiple antigenic reactive sites located predominantly within the B and C subdomains of the COOH-terminus of BP230. J Invest Dermatol. 2000;114(5):998–1004.

Verraes S, et al. Respective contribution of neutrophil elastase and matrix metalloproteinase 9 in the degradation of BP180 (type XVII collagen) in human bullous pemphigoid. J Invest Dermatol. 2001;117(5):1091–6.

Zillikens D, et al. Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. J Invest Dermatol. 1997;109(4):573–9.

Systemic Autoimmune Disease and Premature Atherosclerosis

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Synonyms

Cardiovascular disease (CVD) = atherosclerotic disease; Premature atherosclerosis = accelerated latherosclerosis; Systemic autoimmune disease = rheumatologic disease

Definition

Premature atherosclerosis is the formation of arterial atheromatous plaques at an earlier age than would be expected based on traditional (Framingham) CV risk factors. This is a well-recognized process in systemic autoimmune diseases, most notably rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

Atherosclerosis in RA: Epidemiology, Screening, and Treatment

RA, a disease that affects approximately 1 % of the population, is associated with a 50 % enhanced risk for death secondary to CVD compared to healthy controls, with patients who are seropositive for either rheumatoid factor (RF) activity or antibodies against citrullinated peptides at particular risk (reviewed in Kaplan 2010). Patients with prolonged arthritis have more atherosclerosis than patients of the same age with more recent disease onset. In addition, extra-articular manifestations of RA, usually related to uncontrolled inflammation, are associated with increased CV mortality – suggesting that processes intrinsic to RA pathogenesis play an important role in vascular damage and its clinical consequences (see also ▶ Rheumatoid Arthritis, Clinical Features).

While a practical approach to the detection and treatment of traditional CV risk factors in RA has been suggested (reviewed in Kaplan 2010), consensus guidelines have been, until recently, lacking. In 2010, the European League Against Rheumatism (EULAR) issued recommendations for CV risk management in patients with RA and other forms of inflammatory arthritis – proposing a 1.5-fold multiplication factor to standard CV risk score models in patients with (1) RA for at least 10 years, (2) RF and/or anti-cyclic citrullinated peptide (CCP) positivity, or (3) severe extra-articular features. While there is a general consensus regarding aggressive assessment and management of traditional CV risk factors in RA patients, the role of specialized CV diagnostic testing remains unknown.

As discussed above, there are hints that improved control of disease activity and systemic inflammation in RA can help reduce the atherosclerotic burden. While exposure to classic disease-modifying antirheumatic drugs (like methotrexate, leflunomide, and sulfasalazine) is associated with a reduction in CV risk (reviewed in Kaplan 2010), this "win-win" concept is not as straightforward as it might at first appear – as a number of the medications used to treat RA (including corticosteroids, NSAIDs, and TNF-α blockers) may have competing effects on CV risk. This concept is exemplified by corticosteroids, which on the one hand decrease inflammation but on the other hand promote dyslipidemia, hypertension, and insulin resistance. Regarding biologic therapy, studies in non-RA patients have shown that short-term TNF-α antagonism with infliximab does not improve, and may adversely affect, the clinical condition of patients with moderate-to-severe chronic heart failure. While there are no prospective studies to assess the interplay between biologic therapy and atherosclerosis, the balance of the retrospective literature points toward a protective effect – in terms of both endothelial function and plaque formation (reviewed in Kaplan 2010; see also ▶ Rheumatoid Arthritis, Treatment and ▶ Rheumatoid Arthritis, Biologics in its Treatment).

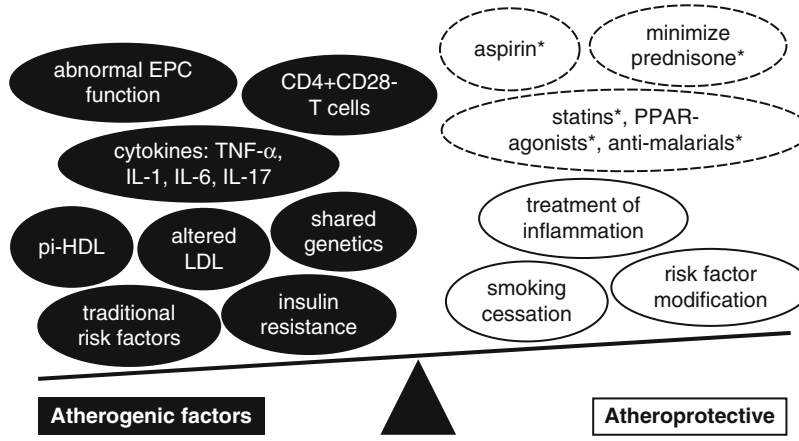
Atherosclerosis in RA: Potential Mechanisms

For many years, striking similarities in the inflammatory and immunologic characteristics of atherosclerosis and RA have been noted. While RA patients do have higher Framingham risk scores than the general population, traditional risk factors are insufficient to entirely explain the increased CV risk in this disease. Many factors have been put forth to explain this enhanced risk (Fig. 1), including metabolic derangements like insulin resistance, family history, altered low-density lipoprotein (LDL) subclass distribution, proinflammatory high-density lipoprotein (HDL), genetic associations, inflammatory cytokines, abnormal lymphocyte populations, and defective endothelial homeostasis (reviewed in Kaplan 2010).

Nevertheless, there is no clear consensus, and the overall story appears to be fairly complex. The most nuanced of the above factors will be discussed below.

Atherosclerosis in RA: Proinflammatory HDL

HDL is considered an atheroprotective factor, facilitating cholesterol efflux from peripheral tissues and protecting endothelial LDL against



Systemic Autoimmune Disease and Premature Atherosclerosis, Fig. 1 Potential atherogenic and atheroprotective factors in RA. On the *left side* of the schematic are factors that are potentially atherogenic in RA. On the *right* are strategies that may be atheroprotective. The items marked with an asterisk (*) are of theoretical benefit, but insufficient data exists to make these standard recommendations. Abbreviations: *pi-HDL* proinflammatory HDL

oxidation. However, HDL undergoes a number of changes in content when exposed to an inflammatory milieu; these include a reduction in apoA1 and paraoxonase (a potent antioxidant) and an increase in serum amyloid A and atherogenic lipids such as nonesterified cholesterol (reviewed in Norata et al. 2011). This is sometimes referred to as proinflammatory HDL as it loses its ability to suppress inflammation at the level of the endothelium. Individuals with both RA and SLE (as discussed below) are more likely to have HDL with a proinflammatory phenotype, which, as expected, correlates with increased levels of oxidized LDL (oxLDL) (McMahon et al. 2006).

Atherosclerosis in RA: Genetic Associations

One of the potential genetic influences linking RA and atherosclerosis involves a polymorphism in the type III promoter of the MHC class II transactivator (MHC2TA). The polymorphism results in differential MHC expression in vitro and has been associated with increased susceptibility to both RA and myocardial infarction (MI). Shared epitope (SE) alleles (from the HLA-DRB1 genotype) have also come under scrutiny with several studies linking the SE, sometimes in combination with other factors, to coronary artery disease; a mechanism by which the SE leads to vascular damage has not been proposed. Single papers have also implicated polymorphisms in IL-6, TNF-α, plasminogen activator inhibitor I, coagulation factor XIII, and TNF receptor type II as risk factors for CVD (or related disease features such as dyslipidemia) in RA patients (reviewed in Kaplan 2010; See also ▶ Rheumatoid Arthritis, Genetics).

Atherosclerosis in RA: Inflammatory Cytokines

Myriad studies have considered the correlation between markers of inflammation – such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hsCRP, TNF-α, IL-1, IL-6, and IL-17 – and atherosclerosis in RA patients (reviewed in Sattar et al. 2003). At this point, the balance of the data points toward a positive correlation between all of the aforementioned biomarkers and CVD in RA. While the mechanism by which the inflammation of RA promotes atherosclerosis is not entirely clear, it presumably parallels emerging data regarding the general role of inflammation in atherosclerosis (reviewed in Hansson and Hermansson 2011; see also ▶ Atherosclerosis and Cytokines).

Atherosclerosis in RA: Abnormal Lymphocyte Populations

In terms of the adaptive immune system, T cells provide the best studied link between RA and atherosclerosis. The most referenced story involves an unusual and expanded population of oligoclonal $CD4^+CD28^-$ T cells present in the blood of patients with RA, as well as patients with unstable angina and no inflammatory arthritis. These cells have enhanced cytolytic and survival potential and can infiltrate unstable plaques. Patients with RA with persistent $CD4^+CD28^-$ T-cell expansion have increased preclinical atherosclerotic changes, including endothelial dysfunction (reviewed in Kaplan 2010). Also warranting further study is the emerging role of T_H17 cells and IL-17 (both critical for RA pathogenesis) in atherosclerosis, as murine studies have heretofore hinted at both atherogenic and atheroprotective phenotypes (see also ▶ Lymphocytes in Atherosclerosis).

Atherosclerosis in RA: Defective Endothelial Homeostasis

A final point involves the disruption of the normal balance between endothelial damage and repair in RA. Endothelial progenitor cells (EPCs) are present in the circulation of patients with CV damage and are released from the bone marrow in increased numbers during acute vascular injury. Indeed, data (independent of RA) has correlated reduced EPC numbers and abnormal EPC function with atherosclerosis and future CV events. It has also been shown that EPC numbers are reduced in the circulation of patients with active RA (reviewed in Kaplan 2010), an effect which may be rescued by anti-inflammatory treatment, such as anti-TNF-α therapy.

Atherosclerosis in Other Inflammatory Arthritides

The majority of the focus has been on two diseases classified as seronegative spondyloarthropathies, namely, ankylosing spondylitis (AS) and psoriatic arthritis (PsA) (see also ▶ Spondyloarthritis: Ankylosing Spondylitis and ▶ Spondyloarthritis: Psoriatic Arthritis). In terms of CV manifestations, the risk of atherosclerosis is usually assumed to be similar to that for RA – which is more amenable to study and formulation of clinical guidelines given large, well-established cohorts.

One potential confounder to the evaluation of CVD in the spondyloarthropathies is that AS, in particular, is commonly associated with aortic root disease – an inflammatory sclerosis involving all three layers of the aortic wall which differs markedly from endothelial-based atherosclerosis. Aortic root disease is frequently associated with aortic regurgitation and may be detected in as many as 61 % of AS patients by echocardiography (reviewed in Vinsonneau et al. 2008). Mitral regurgitation, atrioventricular conduction abnormalities, myocardial inflammation, and pericardial involvement have also been described.

Up to 50 % of deaths in AS patients may be attributable to CVD, although at least some of this burden is borne by non-atherosclerotic disease (reviewed in Peters et al. 2004). In terms of conventional CV risk factors, the most reproduced data involves low circulating levels of HDL in AS patients as compared to controls, which inversely correlate with IL-6 and CRP levels; in contrast, there are no definitive data to implicate other risk factors such as hypertension, smoking, or decreased physical activity. Studies of preclinical vascular function in AS are limited by sample size and are much less definitive than for RA. Nevertheless, individual studies – showing abnormal vascular function which improves with anti-inflammatory treatment (i.e., TNF-α blockers) – are reminiscent of the pattern seen in RA. Overall, expert consensus suggests that AS should be considered on par with RA in terms of accelerated atherosclerosis.

More so than in patients with AS and RA, PsA patients have an increased prevalence of traditional CV risk factors (reviewed in Ramonda et al. 2011). In terms of preclinical disease, a significant increase in carotid intima-media thickness (CIMT) and an impairment of flow-mediated vasodilatation (FMD) have been observed in PsA patients without clinically evident CVD. And, similar to what has been described for

AS and RA, anti-inflammatory treatment improves vascular parameters such as aortic stiffness. Presently, the consensus is that accelerated atherosclerosis is a part of PsA. Whether traditional risk factors play a bigger role in PsA than in the other inflammatory arthritides is possible, although has not been definitively proven.

Atherosclerosis in Psoriasis

This topic is closely related to the above discussion regarding PsA. Even in the absence of inflammatory arthritis, psoriatic skin disease itself is strongly associated with CVD, a link that is partly explained by the prevalence of comorbid diseases including the metabolic syndrome. Because psoriasis is a common disease affecting 1–3 % of the white population, epidemiologic data is relatively robust and supports an increased relative risk for MI in patients with psoriasis compared to controls, even after controlling for traditional risk factors (reviewed in Gelfand et al. 2006). The presumed pathophysiology parallels that for RA and inflammatory arthritis above (see also ▶ Psoriasis).

Atherosclerosis in SLE: Epidemiology and Treatment

SLE, a multisystem autoimmune disease that primarily affects women of childbearing age, is characterized by aberrant autoantibody and immune complex (IC) formation, as well as a markedly heterogeneous clinical phenotype (see also ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis). Dating back to the 1970s, a so-called bimodal pattern of mortality was recognized, with early deaths attributable to disease activity and complications of treatment, such as infection, and later deaths largely secondary to CVD. The frequency of premature CVD, especially when considered in young females, is striking and may be as high as 50-fold depending on the study and the specific outcome measure. Further, a significant percentage of patients with SLE have evidence of endothelial dysfunction and subclinical vascular disease (i.e., increased CIMT), while as many as 40 % have evidence of asymptomatic myocardial perfusion abnormalities (reviewed in Kaplan 2009). Traditional risk factors are insufficient to explain the risk of CVD in SLE patients, with both duration of SLE and the amount of lupus-attributable damage predictive of atherosclerosis. While all-cause mortality in SLE has improved significantly with improved immunosuppressive treatment, death secondary to CVD has yet to show a decline.

Presently, there are no consensus guidelines on the management of CVD in SLE, although an assessment of traditional risk factors should begin soon after diagnosis. Despite hints that some SLE medications like antimalarials and mycophenolate mofetil may be vasculoprotective, there is no current indication to employ these agents beyond their typical role in SLE disease management. While the data is probably less clear-cut than in RA, there is certainly a reason to hypothesize (as discussed below) that improved disease control will have an overall atheroprotective effect.

Atherosclerosis in SLE: Potential Mechanisms

The etiology of accelerated atherosclerosis in SLE likely differs from that for RA and other inflammatory arthropathies; in particular, the role of "high-grade" inflammation is less important in lupus, highlighted by the distinct cytokine milieu of SLE in which CRP is classically suppressed during disease flares. A number of unique factors may play a role in the accelerated CVD of SLE – of these, type I interferons (IFNs), like IFN-α, are especially important. Other factors including proinflammatory lipids, potentially pathogenic autoantibodies, and the emerging role of platelets and neutrophils will also be discussed here.

Atherosclerosis in SLE: Defective Endothelial Homeostasis

As endothelial cell (EC) death is likely a critical early event in plaque development, proper repair of damaged endothelium is assumed to be atheroprotective. Because the proliferation of

mature ECs plays a relatively limited role in regeneration, circulating cells, both the aforementioned bone marrow-derived EPCs and so-called myeloid circulating angiogenic cells (CACs), are critically important for endothelial health. Similar to RA (and to other conditions with a high risk of atherosclerosis), patients with SLE have lower numbers of circulating EPCs, with impaired ability to differentiate into mature ECs and to incorporate into vascular structures; this is true even in patients without a history of CV events or traditional CV risk factors.

Atherosclerosis in SLE: Type I Interferons

In recent years, it has been suggested that IFN-α (possibly in concert with other type I IFNs) is a principle player in the abnormal endothelial repair of SLE. Type I IFNs (including 15 subtypes of IFN-α, one IFN-β, one IFN-ω, and, in some species, several subtypes of IFN-τ) are important cytokines in host defense – engaging in a number of important processes including growth and differentiation, induction of other cytokines, and stimulation of different immune effector cells. Recently, IFN-α has received considerable interest as a mediator of SLE pathogenesis, as gene expression studies have repeatedly demonstrated elevated expression of type I IFN-regulated genes in lupus peripheral blood mononuclear cells (PBMCs) and in some SLE animal models (reviewed in Kaplan and Salmon 2011).

Building on data from the cancer literature – where type I IFNs have been shown to have potent antiangiogenic properties – investigators have recently considered the role of IFN-α in SLE vasculopathy (Fig. 2). Indeed, IFN-α induces EPC/CAC apoptosis and skews myeloid CACs toward nonangiogenic phenotypes such as dendritic cells (reviewed in Kaplan and Salmon 2011). Further, neutralization of type I IFN pathways completely restores a normal EPC/CAC phenotype in SLE in vitro. The IFN-α antiangiogenic signature is at least partially attributable to repression of IL-1 pathways via upregulation of IL-1 receptor antagonist (IL-1Ra) and downregulation of the angiogenic molecule vascular endothelial growth factor (VEGF). Indeed, this pattern is appreciated in SLE patients who demonstrate vascular rarefaction, repression of VEGF, and increases in IL-1Ra in both renal blood vessels and serum. Cyclic injury and failed repair could allow initiation and expansion of vascular lesions during disease flares, ultimately predisposing to atheroma formation.

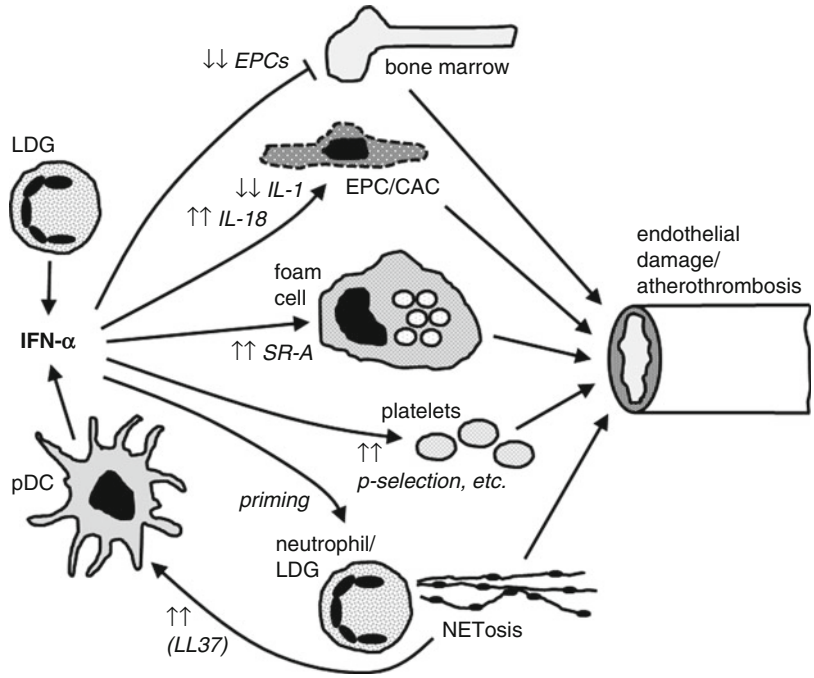
Potentially deleterious links between type I IFNs and the vasculature continue to be described. For example, IFN-α sensitizes the antigen-presenting cells of plaque tissue to bacterial lipopolysaccharide by upregulating TLR4, thereby intensifying TNF-α, IL-12, and matrix metalloproteinase-9 production – all of which have been implicated in plaque destabilization (Niessner et al. 2007). IFN-β treatment accelerates the formation of atherosclerotic lesions in mouse models, while blockade of type I IFN signaling in myeloid cells inhibits lesion development and protects against lesional accumulation of macrophages (Goossens et al. 2010). Further, IFN-α upregulates scavenger receptor A, thereby priming macrophages for oxLDL uptake and foam cell formation (reviewed in Kaplan and Salmon 2011). Finally, the effect of IFN-α on platelets and neutrophils has recently been explored and will be considered in more detail below.

Atherosclerosis in SLE: Proinflammatory HDL

Even more so than patients with RA, patients with SLE have proinflammatory HDL (20 % of patients vs. 45 %) which correlates with increased oxLDL formation, prevalence of carotid plaque, and higher CIMT (McMahon et al. 2006). Proinflammatory HDL has also been appreciated in murine models of SLE where treatment with apoA1 mimetics is currently under investigation (reviewed in Norata et al. 2011).

Atherosclerosis in SLE: Autoantibodies

The role of autoantibodies in the accelerated atherosclerosis of SLE is a complex topic (reviewed in Narshi et al. 2011). Early reports from the 1980s demonstrated IgG-containing ICs within atherosclerotic plaques, although such clear-cut data has not necessarily been reproduced. There are also hints that ICs have biologic activity at the



Systemic Autoimmune Disease and Premature Atherosclerosis, Fig. 2 Potential roles for IFN-α in atherosclerosis. Plasmacytoid dendritic cells (*pDCs*) and low-density granulocytes (*LDGs*) are the primary sources of IFN-α in SLE. IFN-α downregulates EPC/CAC numbers and function, while upregulating foam cell formation, platelet activity, and NET formation (*NETosis*). NETosis, and the antibacterial peptide LL37, can stimulate additional IFN-α production from pDCs. Ultimately, the imbalance between vascular damage and repair leads to a dysfunctional endothelium and potentially atherothrombosis. Other abbreviations: *SR-A* scavenger receptor A

level of the endothelium, although a definitive contribution of ICs to atherosclerosis has yet to be proven.

There has been considerable focus on autoantibodies directed against structural endothelial proteins, so-called anti-EC antibodies, especially in the context of lupus vasculitis. While there are no definitive links to atherosclerosis, anti-EC antibodies may mediate apoptosis through an Fc receptor-independent mechanism, while also inducing a proinflammatory phenotype through NF-κB-dependent pathways.

While there is murine data to support a pathogenic role of IgG anti-oxLDL antibodies, the bulk of the human data (especially for natural IgM antibodies) points toward an atheroprotective effect. Data is further confounded by the fact that oxLDL forms complexes with β_2-glycoprotein 1 (β_2GP1), the key antigen of the antiphospholipid antibody syndrome (APS), making the true target of potentially pathogenic antibodies less clear. Other potentially relevant atherogenic autoantibodies include those directed against HDL, apoA1, HSP 60/65, and unmodified LDL. Finally, an atherogenic role for antiphospholipid antibodies (aPLs) – found in 20–30 % of lupus patients – has been suggested, although epidemiologic data linking aPLs to atherosclerosis in SLE is mixed. These antibodies will be considered in more detail in the section pertaining to primary APS (see also ► Autoantibodies to Endothelial Cells).

Atherosclerosis in SLE: Platelets

Platelets from patients with SLE have an activated phenotype. As mentioned above, an IFN-α signature has recently been described in platelets isolated from SLE patients, correlating with both

platelet activation and a history of CVD (reviewed in Kaplan and Salmon 2011). CD40L expressed on the platelet surface may also modulate plasmacytoid dendritic cell activity and resultant IFN-α production; in this model, platelet depletion reduced SLE activity and disease damage in murine lupus. The mechanism by which platelets contribute to CVD in SLE awaits further study, although it is likely (drawing parallels to the emerging role for platelets in atherosclerosis) that platelets modulate inflammation at the level of the lupus endothelium, with potential implications for the employment of antiplatelet drugs to prevent CVD in SLE (see also ▶ Platelets, Atherosclerosis, and Immunity).

Atherosclerosis in SLE: Neutrophils

Since 2010, a number of groups have recognized, and begun to explore, the role of so-called neutrophil extracellular traps (NETs) in SLE pathogenesis (reviewed in Kaplan 2011). NET formation is a recently recognized form of neutrophil cell death, distinct from apoptosis and necrosis, by which strands of chromatin "beaded" with granule-derived antimicrobial peptides are extruded from the cell. Certainly in sepsis models, and now in one SLE model, NET release damages the endothelium; at this point, the role of NETs in atherosclerosis is unknown (see also ▶ Neutrophils in Endothelial Damage).

Other Diseases

At this point, accelerated atherosclerosis is a well-accepted manifestation of the inflammatory arthritides (RA, AS, and PsA) and SLE. There are hints that patients with other systemic autoimmune diseases (as will be discussed below) may also develop premature atherosclerosis.

Atherosclerosis in Primary Antiphospholipid Antibody Syndrome (APS)

APS is a syndrome which, by definition, requires either vascular thrombosis or pregnancy loss *and* a durably positive lab test for either anticardiolipin antibodies, anti-β_2GP1 antibodies, or a functional lupus anticoagulant (see also ▶ Antiphospholipid Syndrome, Clinical Manifestations). Of these, the majority of basic science work over the past decade has focused on antibodies to β_2GP1, an apolipoprotein which binds to negatively charged lipids (such as phosphatidylserine and oxLDL). Anti-β_2GP1 antibodies produce a procoagulant state, probably by interacting with endothelial and platelet surfaces, while also modulating the coagulation cascade. Both arterial and venous thromboses have been described in APS, and the syndrome is sometimes associated with other systemic autoimmune diseases, classically SLE. When the syndrome is found in isolation, it is referred to as primary APS. In the largest APS cohort published to date, deep venous thrombosis was observed in 32 % of patients, stroke in 13 %, pulmonary embolus in 9 %, and MI in 3 %.

Since arterial thrombosis is a prominent part of the disease process (as well as the diagnostic criteria), an event-based assessment of atherosclerotic disease is not straightforward. Studies have therefore focused on markers of preclinical disease (CIMT/FMD) in an attempt to understand whether the typical endothelial alterations seen in classic early atherosclerosis (as in SLE) are present in primary APS. The results have been mixed and confounded by the inclusion of patients with secondary APS. Nevertheless, a recent consideration of the topic (reviewed in Ames et al. 2009) found that the majority of published studies do show a significant increase in CIMT in primary APS patients as compared to controls. Further, because these patients are typically young, the differences are not explained by traditional risk factors. A definitive statistical consideration of the topic (which also takes into account studies of FMD in APS patients) has not been performed.

Atherosclerosis in Primary Sjogren's Syndrome (SS)

SS is a systemic connective tissue disease resulting in lymphocytic infiltration of exocrine

glands and other tissues (see also ▶ Sjögren's Syndrome). Like APS, a secondary form of SS is well recognized and can be associated with diseases like RA and SLE. There is no comprehensive review of the association between primary SS and atherosclerosis in the literature, with only a few published studies available.

A higher-than-expected prevalence of CIMT has been described, associated with anti-SSA antibodies and leukopenia (Vaudo et al. 2005). Furthermore, endothelium-independent vasodilation is impaired in Sjogren's syndrome – the significance of this finding is not entirely clear, although the authors suggest that it may point toward a smooth muscle-specific defect in patients with primary SS (Gerli et al. 2010). Presently, there is insufficient data to suggest that primary SS is an independent risk factor for atherosclerosis.

Atherosclerosis in Systemic Sclerosis (SSc)

SSc is a chronic disease of unknown etiology, characterized by enhanced fibrosis and microvascular abnormalities (see also ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations). Because SSc is characterized by calcification, vasculopathy, and endothelial wall damage, it has been hypothesized that these patients may be at increased risk for atherosclerosis (Au et al. 2011). Further, over the past four decades, death rates owing to SSc-related complications have gradually decreased, while death rates due to atherosclerotic CVD or cerebrovascular disease have substantially increased; indeed, CV-related deaths are responsible for a 20–30 % mortality rate in SSc patients. Analysis of traditional risk factors has primarily focused on lipid profiles, with studies looking at both proinflammatory HDL and oxLDL; results have not been consistent and have often not reached statistical significance (reviewed in Nussinovitch and Shoenfeld 2011).

It should be noted that other cardiac manifestations of SSc, such as myocardial fibrosis due to ischemia-reperfusion injury, have been noted. Also, while endothelial dysfunction in the capillaries and arterioles is a common and characteristic finding in SSc, there is controversy as to whether this involvement extends to medium and large vessels.

A recent meta-analysis focusing on the preclinical parameters of CIMT and FMD did suggest dysfunction in scleroderma patients (Au et al. 2011). The mean CIMT was associated with SSc disease duration and was comparable to data from patients with RA, diabetes, and familial hypercholesterolemia. Overall, there is no consensus opinion on this topic with recent reviews coming to differing conclusions as to whether there is true accelerated macrovascular disease in SSc, as has been proven for RA and SLE (Au et al. 2011; Nussinovitch and Shoenfeld 2011). Further study is clearly needed.

Atherosclerosis in the Idiopathic Inflammatory Myopathies (IIMs)

IIMs represent a heterogeneous group of systemic autoimmune diseases characterized by chronic muscle weakness and inflammatory cell infiltrates in skeletal muscle; polymyositis and dermatomyositis are the most common IIMs (see also ▶ Myositis: Polymyositis, Dermatomyositis, Inclusion Body Myositis, and Myositis Autoantibodies). Extramuscular manifestations have been described, and CV manifestations include isolated electrocardiographic changes, valve disease, coronary vasculitis, ischemic abnormalities, heart failure, and myocarditis. The literature contains scant references specifically addressing accelerated atherosclerosis in the IIMs (Soltesz et al. 2009), and no clear consensus has been reached.

Atherosclerosis in Systemic Vasculitis

The clinical manifestations of vasculitis are dependent upon both the location and size of the involved vessels, as well as upon the nature of the inflammatory process (see also ▶ Cutaneous Vasculitis). While vasculitis can occur as a secondary condition (e.g., in infection or RA),

primary idiopathic vasculitides are well described and have a chronic disease course that resembles the systemic autoimmune diseases discussed above.

In the so-called "large-vessel" vasculitides, giant cell arteritis (GCA), and Takayasu arteritis (TA), the vasculitic process is confined to the aorta and its major branches. GCA, typically a disease of individuals over 50 years old, has not been associated with accelerated atherosclerosis (reviewed in Tervaert 2009). In contrast, accelerated atherosclerosis has been documented in TA; this is based on both autopsy data and evaluations of preclinical disease. TA patients with plaques tended to be older and have higher levels of total cholesterol.

The medium-vessel vasculitides include Kawasaki disease (KD) and polyarteritis nodosa (PAN). KD typically affects children younger than 5 years of age, with one-third of patients developing CV complications such as coronary artery dilatation and pericarditis. A study in adolescents with a history of KD and coronary lesions did reveal increased CIMT not explained by alterations in lipid profiles; this result was subsequently confirmed by a larger and more recent study from Hong Kong (reviewed in Tervaert 2009); in contrast, studies of brachial artery FMD have not been as consistent in KD. Although PAN can also potentially result in inflammation of the coronary arteries, the issue of accelerated atherosclerosis in PAN has not been evaluated in the literature.

The small-vessel vasculitides include granulomatosis with polyangiitis (Wegener's), microscopic polyangiitis (MPA), Churg-Strauss syndrome (CSS), Henoch-Schonlein purpura, and essential cryoglobulinemia, among others; disease manifestations are diverse but frequently include glomerulonephritis. ANCA-associated vasculitis (which includes Wegener's, MPA, and CSS) is the only group that has been studied from a CVD perspective. Indeed, by long-term follow-up studies, CVD does appear to be a major cause of mortality in these patients with stroke and MI having odds ratios on the order of 3–4 (reviewed in Tervaert 2009). However, noninvasive testing (CIMT and FMD) results have been mixed with no clear consensus emerging.

Conclusions

Premature atherosclerosis is clearly a manifestation of RA and SLE; both disorders have large well-established patient cohorts that have made this recognition possible. For RA (and inflammatory arthritis as a group), "high-grade" inflammation seems to be an important part of the pathogenesis of atherosclerosis, with many interesting parallels drawn between the inflammatory milieu (both local and systemic) of RA and atherosclerosis. For SLE, the situation may be more complex, as type I IFNs clearly contribute to lupus endothelial dysfunction; autoantibodies and proinflammatory lipids may also play a role. Management guidelines for CVD in RA and SLE are just beginning to emerge – at this point, guidelines focus on identification and treatment of traditional risk factors, while optimizing control of disease activity.

The situation is less clear for the other systemic autoimmune diseases. For almost all of these, reports and small series can be found which point toward an increased frequency of either CV events or preclinical disease. However, series that do not show an association also exist, and, in general, definitive meta-analyses have not been performed. At this point, it is probably prudent to consider all of the systemic autoimmune diseases as potentially carrying the risk of accelerated atherosclerosis, with the idea that this will heighten the clinician's attention to treatable traditional risk factors.

References

Ames PR, Margarita A, Alves JD. Antiphospholipid antibodies and atherosclerosis: insights from systemic lupus erythematosus and primary antiphospholipid syndrome. Clin Rev Allergy Immunol. 2009;37:29–35.

Au K, Singh MK, Bodukam V, Bae S, Maranian P, Ogawa R, Spiegel B, McMahon M, Hahn B, Khanna D. Atherosclerosis in systemic sclerosis: a systematic review and meta-analysis. Arthritis Rheum. 2011;63:2078–90.

Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB. Risk of myocardial infarction in patients with psoriasis. JAMA. 2006;296:1735–41.

Gerli R, Vaudo G, Bocci EB, Schillaci G, Alunno A, Luccioli F, Hijazi R, Mannarino E, Shoenfeld Y.

Functional impairment of the arterial wall in primary Sjogren's syndrome: combined action of immunologic and inflammatory factors. Arthritis Care Res (Hoboken). 2010;62:712–8.
Goossens P, Gijbels MJ, Zernecke A, Eijgelaar W, Vergouwe MN, van der Made I, Vanderlocht J, Beckers L, Buurman WA, Daemen MJ, et al. Myeloid type I interferon signaling promotes atherosclerosis by stimulating macrophage recruitment to lesions. Cell Metab. 2010;12:142–53.
Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol. 2011;12:204–12.
Kaplan MJ. Premature vascular damage in systemic lupus erythematosus. Autoimmunity. 2009;42:580–6.
Kaplan MJ. Cardiovascular complications of rheumatoid arthritis: assessment, prevention, and treatment. Rheum Dis Clin North Am. 2010;36:405–26.
Kaplan MJ. Neutrophils in the pathogenesis and manifestations of SLE. Nat Rev Rheumatol. 2011;7:691–9.
Kaplan MJ, Salmon JE. How does interferon-alpha insult the vasculature? Let me count the ways. Arthritis Rheum. 2011;63:334–6.
McMahon M, Grossman J, FitzGerald J, Dahlin-Lee E, Wallace DJ, Thong BY, Badsha H, Kalunian K, Charles C, Navab M, et al. Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum. 2006;54:2541–9.
Narshi CB, Giles IP, Rahman A. The endothelium: an interface between autoimmunity and atherosclerosis in systemic lupus erythematosus? Lupus. 2011;20:5–13.
Niessner A, Shin MS, Pryshchep O, Goronzy JJ, Chaikof EL, Weyand CM. Synergistic proinflammatory effects of the antiviral cytokine interferon-alpha and Toll-like receptor 4 ligands in the atherosclerotic plaque. Circulation. 2007;116:2043–52.
Norata GD, Pirillo A, Ammirati E, Catapano AL. Emerging role of high density lipoproteins as a player in the immune system. Atherosclerosis. 2011;220(1):11–21.
Nussinovitch U, Shoenfeld Y. Atherosclerosis and macrovascular involvement in systemic sclerosis: myth or reality. Autoimmun Rev. 2011;10:259–66.
Peters MJ, van der Horst-Bruinsma IE, Dijkmans BA, Nurmohamed MT. Cardiovascular risk profile of patients with spondylarthropathies, particularly ankylosing spondylitis and psoriatic arthritis. Semin Arthritis Rheum. 2004;34:585–92.
Ramonda R, Lo Nigro A, Modesti V, Nalotto L, Musacchio E, Iaccarino L, Punzi L, Doria A. Atherosclerosis in psoriatic arthritis. Autoimmun Rev. 2011;10(12):773–8.
Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003;108:2957–63.
Soltesz P, Der H, Kerekes G, Szodoray P, Szucs G, Danko K, Shoenfeld Y, Szegedi G, Szekanecz Z. A comparative study of arterial stiffness, flow-mediated vasodilation of the brachial artery, and the thickness of the carotid artery intima-media in patients with systemic autoimmune diseases. Clin Rheumatol. 2009;28:655–62.
Tervaert JW. Translational mini-review series on immunology of vascular disease: accelerated atherosclerosis in vasculitis. Clin Exp Immunol. 2009;156:377–85.
Vaudo G, Bocci EB, Shoenfeld Y, Schillaci G, Wu R, Del Papa N, Vitali C, Delle Monache F, Marchesi S, Mannarino E, et al. Precocious intima-media thickening in patients with primary Sjogren's syndrome. Arthritis Rheum. 2005;52:3890–7.
Vinsonneau U, Brondex A, Mansourati J, Saraux A, Cornily JC, Arles F, Godon P, Quiniou G. Cardiovascular disease in patients with spondyloarthropathies. Joint Bone Spine. 2008;75:18–21.

Systemic Lupus Erythematosus, Animal Models

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Synonyms

Lupus models; Lupus-prone mice

Definition

Certain animals, mostly mice, are prone to developing autoimmune manifestations and organ damage reminiscent of human SLE. Such animals are extensively used to understand mechanisms of disease pathogenesis and conduct preclinical therapeutic trials.

Introduction

SLE animal models have greatly facilitated the study of lupus and helped to develop rational

new treatments. In addition, lupus-prone mice have served as important tools in the study of genes involved in the expression of autoimmunity. Genes that facilitate or inhibit disease have been identified, and these have in turn facilitated the study on immunogenetics and immunopathogenesis of lupus (Tsokos 2011). Etiology of SLE is heterogeneous and complicated, but animal models bring a rational understanding of disease pathogenesis. Mouse models can be grouped into three types: spontaneous, gene manipulation derived, and induced (Kono and Theofilopoulos 2011).

Spontaneous Models

Hybrids with New Zealand White (H-2^z), (NZBxNZW) F1 mice develop systemic autoimmune disease with high titer of anti-DNA antibodies and severe glomerulonephritis (GN) that becomes apparent at 5–6 months of age. The average lifespan of these mice is 8 months for females and 13 months for males (Cohen and Maldonado 2003).

NZM2328 and NZM2410 mice: These strains derive from New Zealand Mixed (NZM) mice generated by (NZBxNZW) F1 and NZW backcross and sib mating. They were found to develop lupus-like disease. Importantly, three lupus susceptibility loci, *Sle1-3*, were identified by lupus linkage analysis of NZM2410 mice. Study of these mice has revealed variations in many genes in these loci to be directly associated with human SLE. *Sle1b* corresponds to polymorphisms in four signaling lymphocytic activation molecule (SLAM) family member genes including Ly108, which is directly implicated in the regulation of B-cell tolerance. Variants of SLAMF3 (Ly9) and SLAMF4 (CD244) have been associated with human SLE and RA.

MRL-Fas$^{lpr/lpr}$ (MRL/lpr) mice: They show accelerated autoimmune disease characterized by severe lymphoadenopathy due to the accumulation of $CD3^+CD4^-CD8^-B220^+$ (double-negative) T cells. Disease onset with severe dermatitis and/or lymphoadenopathy is seen from 10 to 12 weeks and death occurs at around 25 weeks. Disease is severe in female and the average lifespan for female MRL/*lpr* mice ranges from 17 to 35 weeks, depending on the environment. Mice display high concentrations of immunoglobulins including elevated levels of autoantibodies such as ANA, anti-ssDNA, anti-dsDNA, anti-Sm, and rheumatoid factors (Seavey et al. 2011). B cells and T cells from these mice have a defect in apoptosis due to the lack of functional Fas receptor. A mutation of Fas Ligand gene leads to generalized lymphoproliferative disease (gld) similar to that caused by the *lpr* mutation. In humans, defective Fas signaling can lead to the development of autoimmune lymphoproliferative syndrome (ALPS) which shares manifestations with SLE. Expression of the *lpr* mutation in non-autoimmune strains such as C3H/HeJ and C57BL/6 leads to the development of lymphoproliferation and autoantibody production but limited glomerulonephritis in female mice.

BXSB mice develop severe glomerulonephritis and autoantibodies, but the disease is limited to males. The genetic locus responsible for the expression of the disease is located in the Y chromosome and is known as *Yaa* (Y chromosome-accelerated autoimmunity). *Yaa* was first identified from a cross between a C57BL/6 female and an SB/Le male that produced the BXSB hybrid line. BXSB mice develop SLE at much higher incidence and with earlier onset in males compared to females, whereas mice from the reciprocal cross (SB/LE female and B6 male) do not show the same acceleration of disease in males. The disease is heavily dependent on the presence of the H-2^b allele, because its replacement with the H-2^d allele results in prolonged survival. Toll-like receptor-7 (TLR7), a single-stranded RNA-binding innate immune receptor, is a gene responsible for the Y chromosome-linked autoimmune accelerator (Pisitkun et al. 2006).

Mice that develop spontaneous autoimmunity have helped understand the role of hormonal and immunoregulatory influences in autoimmunity, but obvious restraints limit direct transfer of this information to human disease. Human disease develops in individuals with a permissive genetic

background in conjunction with environmental and stress factors, possibly acting over a long period. However, the murine models allow us to analyze disease mechanisms and are very useful to test potential therapies. Moreover, analysis of gene-manipulated mice in these genetic backgrounds enables investigators to identify a number of molecules involved in the development of disease pathogenesis.

Genetically Manipulated Models

Loss of tolerance is a fundamental immunologic abnormality in SLE. Numerous studies in mice with genetic manipulations including gene deletions and transgenes that develop autoantibodies and other features of SLE on non-autoimmune genetic backgrounds have provided great insights into mechanisms that govern tolerance and autoimmunity and suggested novel rational treatments.

Lymphocyte Activation Molecules

PD-1-deficient mice: Programmed death-1 (PD-1) is a member of the CD28 family of receptors and its intracytoplasmic domain defines an immunoreceptor tyrosine-based inhibition motif (ITIM), and when engages with its ligands PD-L1 and PD-L2 delivers a negative signal. Mice lacking the PD-1 gene develop either autoantibody-mediated cardiomyopathy in BALB/c or glomerulonephritis in C57BL/6 background.

Mgat5-deficient mice: Mice with beta-1,6 N-acetylglucosaminyltransferase V (Mgat5) deficiency display decreased glycosylation of T-cell membrane proteins, which prevents galectin binding and thereby disrupts the galectin-glycoprotein lattice, leading to increased clustering of TCR. Increased TCR clustering in these autoimmune mice provides a phenotype comparable with human SLE involving lowered T-cell activation thresholds and increased TCR signaling.

Lyn-deficient mice: A state of B lymphocyte hyperactivity resembling SLE is seen in mice lacking the src-family kinase Lyn. Lyn is an essential inhibitory component on B-cell receptor (BCR) signaling. Negative regulation of the BCR is a complex quantitative trait in which Lyn, the coreceptor CD22, and the tyrosine phosphatase SHP-1 are each limiting elements. Lyn-deficient mice display decreased numbers of mature peripheral B cells, greatly elevated serum IgM and IgA, and production of autoantibodies that cause autoimmune glomerulonephritis reminiscent of SLE. Sustained activation of Lyn in vivo using a targeted gain-of-function mutation ($Lyn^{up/up}$ mice) led to the development of autoantibodies and lethal autoimmune glomerulonephritis.

FcγRIIb-deficient mice: Antigen presented in the context of immune complexes engages not only the BCR but also the FcγRIIb, which results in the phosphorylation of the immunoreceptor tyrosine-based inhibitory motif (ITIM) defined by its intracytoplasmic domain. Recruitment of the phosphatases SHIP (Src homology 2 domain-containing inositol-5-phosphatase) and SHP1 suppresses signaling. FcγRIIb-deficient mice display increased production of autoantibodies, and glomerulonephritis and FcγRIIb deficiency exacerbates autoimmunity in B6/lpr mice.

Ubiquitination-Protein Ligases

Cbl-b-deficient mice: The Cbl-b and Cbl adaptor proteins are E3 ubiquitin ligases that inhibit receptor and non-receptor tyrosine kinases by promoting ubiquitination of signaling molecules. Loss of Cbl-b rescues reduced calcium mobilization of anergic T cells, which was attributed to Cbl-b-mediated regulation of PLCγ-1 phosphorylation. Loss of Cbl-b in mice results in impaired induction of T-cell tolerance both in vitro and in vivo and shows exacerbated autoimmunity. Moreover, B-cell-specific deficiency of Cbl/Cbl-b shows impaired BCR downmodulation and anergy to self-antigen, and develop spontaneous lupus-like disease presenting with anti-dsDNA, ANA, massive leukocytic infiltrates in multiple organs, and immune complex GN.

$Rc3h1^{san/san}$ mice: Roquin (Rc3h1), a RING-type ubiquitin ligase family member, is a negative regulator of the development of follicular helper T (Tfh) cell, a T helper (Th) cell subset specialized for providing help to B cells

in germinal center. This molecule is identified by a novel forward genetic strategy: Male C57BL/6 mice were treated with ethylnitrosourea, a mutagenic agent, and bred the variant genome sequences to homozygosity and screened for autoimmunity. Mice with M199R mutation within the ROQ domain of Roquin were generated by ENU mutagenesis that resulted in ANA, anti-dsDNA, glomerulonephritis, necrotizing hepatitis, anemia, and immune thrombocytopenia. This mouse displays increased germinal center formation and expansion of memory/effector $CD4^+$ T cells, particularly Tfh cells.

Cytokines and Their Receptors

Mice that are deficient in IL-2 and IL-2R have disrupted immunological homeostasis that eventually leads to fatal autoimmune manifestations. Specifically, both IL-2-deficient mice and IL-2Rα-deficient mice develop autoimmune hemolytic anemia and colitis with lymphoproliferation, expansion of effector/memory phenotype T cells, polyclonal hypergammaglobulinemia, and autoantibodies. IL-2Rβ−/− mice likewise develop anemia, splenomegaly, and lymphoadenopathy, but not colitis. Activation-induced cell death (AICD) is central for the elimination of activated autoreactive cells, and this depends on IL-2 signaling. Defective AICD obviously plays a role in the development of autoimmunity. Humans with SLE have defective AICD that appears to be multifunctional: defective TNF-α and IL-2 production (Kammer and Tsokos 2002). IL-2 plays a central role in the development and maintenance of regulatory T (Treg) cells, which have been proven to be of pathogenic relevance in the development of autoimmune disease.

BAFF-transgenic or *TACI-knockout mice*: The tumor necrosis factor/ receptor (TNF/TNFR) system participates in the homeostasis of the immune system in different ways. Among them, TNFSF13B (BAFF, BlyS) and its receptor TNFRSF13B (TACI) is implicated in the development of autoimmune disease. BAFF is critical for B-cell survival and BAFF-transgenic or TACI-knockout mice have a lupus-like disease. Moreover, serum BAFF is elevated in both BWF1 and MRL/*lpr* mice, and blocking BAFF function with a soluble TACI-IgGFc protein can inhibit proteinuria and prolong survival.

Interferon-gamma (IFN-γ-transgenic mice): IFN-γ is required for the development of lupus as this cytokine-deficient lupus-prone animal produces significantly reduced production of autoantibody and develops reduced glomerulonephritis. Transgenic mice with a normal genetic background overexpressing IFN-γ in the epidermis develop lupus-like syndrome with antinuclear antibodies and glomerulonephritis with the deposition of immune complex.

Complement and Complement Receptor Proteins

C1q-deficient mice: Activation of classical pathway typically starts by interaction of C1q with immune complexes. The complement system also has an important role in clearing immune complex from circulation. It can also bind apoptotic cells and helps to eliminate these cells from tissue (Carroll 2004). More than 90 % of individuals with C1q and C1r/C1s deficiency develop an SLE-like disorder, while 10–20 % of individuals with C2 deficiency develop SLE. These phenotypes in humans do not parallel in full those in mice. Deficiency of C1q in C57BL/6 mice does not lead to the development of autoimmunity. By contrast, C1q-deficient MRL/MpJ mice display accelerated disease onset and increased levels of antinuclear antibodies and of glomerulonephritis, particularly in females, which developed severe crescentic glomerulonephritis. Moreover, C1q-deficient mice on B6 × 129 genetic background were shown to develop higher levels of autoantibodies compared to strain-matched controls and to develop glomerulonephritis by 8–10 months of age.

CR1/CR2-deficient mice: The B6/*lpr* mouse develops minor autoimmune features. Deficiency in this strain of the complement receptor 1 and complement receptor 2 (CR1/CR2, CD35/CD21), encoded by Cr2 gene, permits development of intense autoimmune features. These receptors are important in the elimination of B cells that display reactivity

with self-antigens. Patients with SLE have around 50 % lower levels of these receptors on their B cells. MRL/*lpr* mice exhibit lower levels of these receptors on B cells prior to the onset of clinical disease. Also, the congenic interval corresponding to *Sle1c*, one of the SLE susceptibility loci, *Sle1*, contains the Cr2 gene.

C4-deficient mice: Since disruption of the C1q, C4, and CR1/CR2 leads to reduced selection against autoreactive B cells and to impaired humoral responses, C1 and C4 could act through CR1/CR2 to enhance humoral immunity and suppress autoimmunity, but each complement component appears to act independently (Manderson et al. 2004). High titers of spontaneous ANA and SLE-like autoimmunity develop in C4-deficient mice and most male mice but not in Cr2−/− animals. The fact that the clearance of circulating immune complexes is impaired in pre-autoimmune C4−/−, but not Cr2−/−, mice favors the role of nuclear antigen-ANA immune complexes in the development of autoimmune disease.

Clearance of Dead Cells

DNaseI-deficient mice: Effective degradation of nucleotides from dead cells and digestion of cellular components by macrophages allow non-inflammatory clearance and recycle of dead cells. DNaseI is the major nuclease present in the blood, urine, and secretions. DNaseI deficiency in non-autoimmune background mice was reported to increase the incidence of SLE manifestations, including positive ANA, anti-DNA, and immune complex glomerulonephritis. Reduced DNaseI activity is observed in the sera of lupus patients and contributes to SLE susceptibility (Tanaka and Miyake 2007).

MFG-E8-deficient mice: Deficiency in the clearance of apoptotic cells is proposed to be one of the causes of SLE. Unengulfed apoptotic cells are present in the germinal centers of the lymph nodes of some SLE patients, and macrophages from these patients often show a reduced ability to engulf apoptotic cells. Milk fat globule-EGF factor-8 (MFG-E8) protein functions as a bridging protein between phosphatidylserine on apoptotic cells to avb3 or avb5 integrins on phagocytic cells. MFG-E8 is primarily expressed on CD68-positive tangible body macrophages within germinal center. MFG-E8-deficient female mice in B6 × 129 gene background develop SLE-like autoimmune disease with anti-dsDNA, ANA, and glomerulonephritis by 40 weeks of age.

Mer^{KD} mice: TAM family members (Tyro3, Axl, and Mer), which are tyrosine kinase receptors expressed on antigen-presenting cells (APCs), promote clearance of apoptotic cells and mice expressing a kinase-dead mutant of Mer (Mer^{KD}) develop SLE-like autoimmunity. TAM receptors negatively regulate the innate immune reaction and TAM-deficient dendritic cells overproduce IL-6, IFN and TNFα, which might be responsible for the induction of autoimmunity.

Innate Immune Cell Signaling

DNA and RNA in apoptotic material can activate B cells and dendritic cells through TLR9, TLR7, and TLR8. As described above, overexpression of TLR7 is responsible for the development of autoimmune diseases in BXSB mice. These results indicate that TLR signaling is linked to the development of autoimmune disease and aberrant activation of innate immunity may contribute to systemic autoimmune diseases including RA and SLE.

TANK-deficient mice: TANK (also known as I-TRAF) is a TNF-receptor-associated factor (TRAF) – binding protein and binds to TRAF1, 2, 3, 5, and 6, all of which are crucial for TLR signaling. TANK is a negative regulator of proinflammatory cytokine production induced by TLR signaling and TANK-deficient mice spontaneously develop lupus-like autoimmune diseases with fatal glomerulonephritis, ANA, and anti-dsDNA. Autoantibody production in TANK-deficient mice is abrogated by antibiotic treatment or the absence of IL-6 or MyD88, indicating that TANK controls TLR signaling by intestinal commensal microbiota (Kawagoe et al. 2009).

Zc3h12a-deficient mice: Zc3h12a is an RNase activated by TLR signaling that promotes the degradation of mRNA. Zc3h12a-deficient mice have early mortality associated with severe hemolytic anemia, lymphoproliferation, and

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Table 1 Representatives of mice models of systemic autoimmune disease

Strain/name induction method	Target gene	Autoantibody production	Arthritis
Spontaneous disease models			
NZB		+	
NZW		+	
(NZBxNZW)F1		+	
NZM2328		+	
NZM2410		+	
MRL-Fas$^{lpr/lpr}$	Fas mutation	+++	+
BXSB		+	
Gene-manipulation-derived models			
Lymphocyte activation			
	PD-1 KO	+	+
	SHP-1 KO	+	
	Mgat5 KO	+	
	Lyn KO	+	
	FcγRIIb KO	+	
Ubiquitination-protein ligases			
	Cbl-b KO	+	
	Roquin KO	+	
Cytokines and their receptors			
	IL-2/IL-2R KO	+	
	BAFF TG	+	
	IFNγ TG	+	
Complement			
	C1q KO	+	
	C4 KO	+	
Molecules involved in dead cell clearance			
	DNaseI KO	+	
	MGF-E8 KO	+	
	Mer^{KD}	+	
Innate immune cell signaling molecules			
	TANK KO	+	
	Zc3h12a KO	+	
Induced models			
Pristane		+	+
GVHD		+	

ANA with increased number of activated B cells, T cells, and plasma cells due to the excessive cytokine transcription such as IL-6 and IL-12.

Induced Models

Information has been acquired over many years from the study of autoimmunity and SLE-like disease which develops in mice after the injection of immune cells of chemicals.

The *isoprenoid alkane pristane* (2, 6, 10, and 14 tetramethylpentadecane) induces autoantibodies characteristic of SLE, including anti-Sm, anti-dsDNA, and anti-ribosomal P in BALB/c and SJL/J mice. Pristane induces type I IFN production from Ly6C^{hi} monocyte through toll-like receptor-7 (TLR7) and myeloid

differentiation factor-88(MyD88) pathway and triggers autoantibody production. Also, IL-12, IFN-γ, but not IL-4 are involved in the development of pristine-induced lupus, indicating that T helper type 1 (Th1) responses are dominant. For unexplained reasons, the *lpr* and *gld* mutations protect mice from the production of antibodies routinely induced by pristine. In addition to nephritis, hemorrhagic pulmonary capillaritis and arthritis have also been observed in pristine-treated mice. The arthritis symptoms in this model include synovial hyperplasia, periostitis, and progressive marginal erosions. Pristane-induced arthritis is TNFα mediated, as the treatment with neutralizing anti-TNFα antibody ameliorates the arthritis symptoms (Beech et al. 1997).

Graft-versus-host induced models of systemic autoimmunity involve the injection of parent cells into F1 offspring and clarified early events in the induction of autoimmunity. When lymphocytes from DBA mice are transferred into (B6 X DBA) F1 mice, donor $CD4^+$ cells are stimulated by recipient MHC class II cells, which presumably present a chromatin-associated nuclear antigen and produce initially IL-2 and later on IL-4 and IL-10, while the generation of $CD8^+$ CTL cells is silenced. These responses result in chronic GVHD with autoantibody- and immune-complex-mediated glomerulonephritis. When B6/H-2^{bm12} lymphocytes, whose MHC class II locus confers a three amino acid substitution in H-2^b, are transferred into (Carroll 2000) B6 hosts, they also develop chronic GVHD with autoantibodies and GN. This model works equally with opposite donor and recipient.

Conclusion

Animal models of systemic human autoimmune disease have served useful information for understanding autoimmunity. Human systemic autoimmune diseases are highly heterogeneous both at the clinical and pathogenic level to the point that the field is not served properly by lumping them along antedated criteria-counting approaches. A critical review of the animal models (Table 1) has been used to understand disease processes and perform preclinical trials of putative new drugs and biologics.

Cross-References

- ▶ Autoimmune Kidney Disease and Pregnancy
- ▶ Nephritogenic Antibodies in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Genetics
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Beech JT, Siew LK, Ghoraishian M, Stasiuk LM, Elson CJ, Thompson SJ. CD4+ Th2 cells specific for mycobacterial 65-kilodalton heat shock protein protect against pristane-induced arthritis. J Immunol. 1997;159(8):3692–7.

Carroll MC. A protective role for innate immunity in autoimmune disease. Clin Immunol. 2000;95(1 Pt 2): S30–8.

Carroll MC. A protective role for innate immunity in systemic lupus erythematosus. Nat Rev Immunol. 2004;4(10):825–31.

Cohen PL, Maldonado MA. Animal models for SLE. Curr Protoc Immunol. 2003; Chapter 15: Unit 15 20.

Kammer GM, Tsokos GC. Abnormal T lymphocyte signal transduction in systemic lupus erythematosus. Curr Dir Autoimmun. 2002;5:131–50.

Kawagoe T, Takeuchi O, Takabatake Y, Kato H, Isaka Y, Tsujimura T, et al. TANK is a negative regulator of toll-like receptor signaling and is critical for the prevention of autoimmune nephritis. Nat Immunol. 2009;10(9):965–72.

Kono DH, Theofilopoulos AN. Genetics of lupus in mice. In: Lahita RG, Tsokos GC, Buyon J, Koike T, editors. Systemic lupus erythematosus. London: Academic Press; 2011. p. 63–105.

Manderson AP, Botto M, Walport MJ. The role of complement in the development of systemic lupus erythematosus. Annu Rev Immunol. 2004;22:431–56.

Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science. 2006;312(5780):1669–72.

Seavey MM, Lu LD, Stump KL. Animal models of systemic lupus erythematosus (SLE) and ex vivo assay design for drug discovery. Curr Protoc Pharmacol. 2011; Chapter 5: Unit 5 60.
Tanaka M, Miyake Y. Apoptotic cell clearance and autoimmune disorder. Curr Med Chem. 2007;14(27):2892–7.
Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365(22):2110–21.

Systemic Lupus Erythematosus, Autoantibodies

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Synonyms

Antibodies; Anti-DNA antibodies; Antinuclear antibodies; Antiphospholipid antibodies; Anti-spliceosome antibodies; Autoimmunity; Immunoglobulin

Definition

Antibodies that react against self-antigens of the organism that produced them.

Introduction

Systemic lupus erythematosus (SLE) is a heterogeneous disease involving many organs, accompanied by immunological abnormalities, particularly antibodies against self-structures. Over 100 autoantibodies have been related to this disease, though few are of truly diagnostic importance (Isenberg et al. 2007; Wandstrat et al. 2006). However, after more than 50 years of research, their real value in SLE remains only partly understood.

History

The first description of autoantibodies occurred in the early 1900s. Ironically, Paul Ehrlich having postulated the notion of "horror autotoxicus" – implying the unlikely prospect that the body's immune system would cause self-harm, was also the first to describe autoantibodies – antibodies to red blood cells (now known to be associated with hemolytic anemia). Hargreaves first described the lupus erythematosus cell – a polymorphonuclear leucocyte with a large phagocytized inclusion body – in the bone marrow aspirates of patients with SLE and 14 years later, antibodies to nuclear antigens (ANAs), more specifically against double-stranded DNA (anti-dsDNA), were identified (Holman 2011).

In the past 50 years, SLE has been associated with many autoantibodies which react with different components of the cell; however, anti-dsDNA antibodies have become the hallmark of SLE, partly because they can be detected in the serum of 70 % these patients (Isenberg 2004).

Relation to Autoimmunity Diseases

The first tangible proof of the likely pathogenicity of autoantibodies in lupus was reported in 1967 in kidney biopsies from patients with lupus nephritis and since then, many clinical correlations have been reported (reviewed in Isenberg et al. (2007)).

However, their real role in autoimmune diseases is often unclear. For example, antibodies to some self-antigens (e.g., nucleic acids, phospholipids, and erythrocytes) participate in the physiological homeostasis, facilitating the clearance of apoptotic cells and oxidized lipids. In addition, ANAs have been detected in up to 20 % of healthy people, though usually in low titers (Isenberg 2004; Almqvist et al. 2011; Pisetsky 2011). Some authors relate this finding to the difficulty in distinguishing "physiologic" from pathogenic autoantibodies, while others believe it represents an autoimmune predisposition. Furthermore, some lupus antibodies were identified up to 9.4 years before SLE symptoms developed and may also be found in the serum of

patients with other diseases (e.g., multiple myeloma). Moreover, some lupus-prone mice display accelerated disease despite having low titers of anti-dsDNA antibody (Wandstrat et al. 2006; Almqvist et al. 2011; Arbuckle et al. 2003).

Therefore, to be associated definitively with the pathogenicity of SLE, autoantibodies must be able to induce disease after passive transfer; they must be isolated from affected organs and linked to specific genes. Although injection of denaturated DNA into healthy animals does not normally generate anti-dsDNA antibody, when combined with histones and binding proteins, autoantibodies may develop. The passive transference of monoclonal anti-dsDNA autoantibodies also induces glomerulonephritis in some normal mice, and these antibodies have also been found in the glomeruli of patients with active lupus nephritis. Finally, the expression of V-genes in transgenic mice induces high-affinity anti-dsDNA autoantibody production, although their expression in normal mice did not induce autoimmune disease (Almqvist et al. 2011; Pathak and Mohan 2011; Ehrenstein et al. 1995; Rekvig et al. 2012).

Origin of Autoantibodies

Potential Triggering Events

Several studies have provided evidence of the role of genetic and environmental factors in triggering autoantibody development. A wide variety of genetic disturbances in innate and acquired immunity result in deficient clearance of immunostimulatory material and can predispose individuals to SLE (Pisetsky 2011).

Increased expression of Fcγ receptor type I on monocytes has been related to lupus nephritis, and polymorphisms in Fcγ RIIB have been associated with SLE in some ethnic groups. In addition, the familial occurrence of SLE and the lupus concordance rates for homozygotic twins (around 25 %) and for heterozygotic twins (2–3 %) also suggest a susceptible genetic background (Pisetsky and Vrabie 2009).

However, the susceptibility loci involved are heterogeneous and do not explain the presence of autoantibodies in healthy subjects. An environmental trigger a likely stimulus. The worldwide female prevalence of SLE (usually 9:1) suggests a hormonal influence in autoimmune susceptibility, but beside the extensive exposure of nuclear antigens during pregnancy, no other gender-specific events have been identified.

Reports of anti-DNA antibody in patients with infections due to *Escherichia coli* or *Klebsiella spp.* make infectious agents a probable trigger for autoantibody development (Isenberg 2004). In fact, bacterial DNA and polymerases have immunological activity, via host cells' Toll-like receptors (TLR). The TLR-9 is important to the production of anti-dsDNA and type-1 interferon. Interestingly, autoantibodies to U1-ribonucleoprotein (U1-RNP) recognize epitopes with strong homology to Influenza B, Herpes simplex-1, and Epstein Barr virus proteins. Even though polyclonal B-cell activation after an infection could explain autoantibody development, their isotypes (mainly IgG2) differ from those in patients with SLE (primarily IgG1 and IgG3 isotypes) (Pisetsky 2011; Pisetsky and Vrabie 2009).

Models of Autoantibodies Development

Triggered by environmental factors or genetic predisposition, the production of autoantibodies seems to be antigen driven. This requires intracellular antigens, like DNA, to become exposed to the immune system. Apoptosis has been proposed to be the event in which self-antigens are cleaved, becoming surface accessible and thus exposing previously cryptic immunogenic epitopes (Isenberg et al. 2007; Almqvist et al. 2011; Yung and Chan 2008).

Normally, in major necrotic events, "backup" mechanisms ensure the elimination of the dying cells, avoiding their presentation in lymphoid tissues. However, in SLE patients, there is an impaired clearance of self-antigens. Genetic defects for DNase I, serum amyloid protein P, and tyrosine kinase have been linked to the development of antinuclear antibodies in murine studies. Moreover, deficiency of C1q, (that promotes phagocytosis of chromatin) leads to an SLE-like disease and anti-C1q autoantibodies have been associated with lupus nephritis. SLE patients also have smaller and impaired

phagocytic cells with shorter life spans, culminating in lengthy exposure of self-antigens. Apoptotic blebs release microparticles into the blood stream, displaying nucleosomal molecules and promoting immune complex (IC) formation. High concentrations of self-antigens are presented to T- and B-lymphocytes. In healthy subjects, auto-reactive T- and B-cells are efficiently removed in early stages of their development, but in SLE patients, defective checkpoints on pre-B-cell development allow auto-reactive B-cells to survive and engage BCR specific to DNA (Almqvist et al. 2011). Moreover, underlying defects of T-lymphocyte antigen receptor signaling can also lead to the generation of auto-reactive T-cells. DNA derived from activated T helper cells induces the production of anti-dsDNA antibodies, and nucleosomes can stimulate T-cells to produce cytokines and help B-lymphocytes to differentiate. An essential step follows auto-reactive B-cell proliferation – BCR gene rearrangement – switching from IgM low-affinity isotype (found in healthy subjects) to the IgG high-affinity isotype (found in SLE patients). Increased levels of BAFF and Th17 cell subset favor these events in SLE patients. When high-affinity autoantibodies bind to their driving antigen, the FcγR-mediated phagocytosis by dendritic cells induces the production of cytokines (e.g., IL-8, IL-1β, TNF-β, IL-18, and IFN-α) essential for presentation of antigens and clonal expansion of B-cells. This rebound effect is strong proof of the likely pathogenic role of autoantibodies in maintaining and amplifying inflammation (Isenberg et al. 2007; Almqvist et al. 2011; Pathak and Mohan 2011; Yung and Chan 2008; Muñoz et al. 2010; Waldman and Madaio 2005).

Since autoantibodies are present in both patients and healthy people, the onset of clinical symptoms is not simply related to the quantity of autoantibodies but to their acquisition of pathogenic properties.

Pathogenic Features of Antigens and Autoantibodies

Research in the pathogenicity of SLE is mainly focused on the two participants of antigen-antibody interaction: (1) specific immunogenic characteristics of a limited subset of self-antigens and (2) intrinsic pathogenic properties of autoantibodies.

Specific Immunogenic Characteristics of Self-Antigens in SLE

The development of autoantibodies to a limited subset of self-antigens, mostly DNA- and RNA-associated macromolecules, suggests that, in SLE patients, these particles may be more immunogenic than in healthy subjects. Antibodies to dsDNA are linked to renal involvement unlike those to single-stranded DNA, confirming the importance of the structure of the target. Moreover, sequences of DNA enriched with particular oligonucleotides provide strong ligands to intracellular TLR-9 and -7, intensifying inflammatory cytokines production and B-cell proliferation. Anti-dsDNA antibody seems to bind a specific sequence in the *N*-methyl D-aspartate receptor (NMDAR), a neuronal glutamate receptor, in patients with neuropsychiatric lupus. In contrast, substitution of arginine for glycine in the sequence of beta-2-glycoprotein-I (B2GPI) is related to the pathogenicity of antiphospholipid antibody syndrome (APS) (Isenberg et al. 2007; Rekvig et al. 2012; Núñez-Álvarez and Cabiedes 2011).

Intrinsic Pathogenic Properties of Autoantibodies in SLE

More recently, the findings of specific properties of autoantibodies in SLE patients have provided information about their role as active pathogenic agents.

Immunoglobulin Isotype and Physical Properties While healthy individuals have low-affinity IgM isotype anti-dsDNA autoantibody, SLE patients display high-affinity IgG isotype autoantibodies. In fact, the positively charged IgG2 and IgG3 subclasses have been associated to lupus nephritis, suggesting that during disease development, autoantibodies undergo isotype switch and affinity maturation (Pisetsky and Vrabie 2009; Muñoz et al. 2010).

Antiphospholipid antibodies have also been found in healthy subjects and patients with infectious and neoplastic diseases, who do not develop APS. A switch from the IgM isotype (that does not bind B2GPI) to the IgG isotype seems to occur in patients. Interestingly, high-affinity may determine which autoantibodies induce glomerulonephritis in mice models (Ehrenstein et al. 1995).

Specific Complementarity Determining Regions Besides their isotype, the amino acid sequences in the variable regions of antibodies can also induce their high avidity. Arginine, asparagine and lysine were found in high percentages in the complementarity determining regions (CDR) of anti-dsDNA antibody, probably by somatic hypermutation. The specific position of these residues within the sequence was also critical to increase antigen affinity, suggesting that during B-cell expansion, the clones containing arginine in their CDR-VH genes would develop into anti-DNA antibody producers (Isenberg et al. 2007; Rahman et al. 2002).

Cross-Reactivity The ability of some antibodies to bind to several host molecules may influence pathogenicity. In fact, negatively charged structures like alpha-actinin, laminin, heparan sulfate, and type-IV collagen have been suggested as glomerular targets for cationic charged anti-dsDNA antibodies, leading to direct damage and local IC formation. The intrinsic features of autoantibodies seem to be linked to the pathogenic role of autoantibodies in SLE and may also explain the differential autoantibody accumulation in target organs and why some autoantibodies are more prone to induce IC formation than others (Yung and Chan 2008; Waldman and Madaio 2005).

Mechanisms of Disease

The precise mechanism by which autoantibodies cause tissue inflammation and damage in systemic lupus erythematosus remains uncertain, but four main pathways have been proposed (Rekvig et al. 2012):

1. Induction of circulating immune complexes that deposit in target organs and activate complement and inflammation
2. Penetration into living cells
3. Binding to cell surface promoting cytolysis or cytotoxicity – by anti-Ro/La, anti-P ribosomal, antilymphocyte, antierythrocyte, and antiphospholipid antibodies
4. Cross-reactivity with extracellular molecules (like heparan sulfate and fragmented chromatin) – by anti-chromatin, anti-dsDNA, and antiphospholipid antibodies

Deposition of Circulating Immune Complex in Target Organs

In active disease, patients have higher levels of circulating IC compared to inactive disease and their deposition in tissues may explain the decrease in anti-dsDNA antibody levels observed in some patients before SLE flares occur. In addition, deficiencies of C4a (which prevents IC precipitation) and reduced expression of CR1 (a complement-binding receptor that clears ICs) were associated with the development of lupus nephritis. However, the concentration of circulating ICs in SLE patients is relatively low and it has been hard to demonstrate their passive glomerular trapping as the primary event for disease. For example, the administration of preformed ICs to healthy mice did not result in glomerular deposition or clinical disease. Current opinion is that IC formation occurs in situ, rather than in circulation, probably following deposition of chromatin or exposure of nucleosomes in target tissues (Rekvig et al. 2012; Yung and Chan 2008; Åhlin et al. 2012).

Penetration into Living Cells

It was long presumed that anti-DNA antibodies could only bind to extracellular (post-apoptotic) chromatin and that the findings of immunoglobulins in Hargreaves' cells and glomerular deposits were artifacts of fixation techniques. However, since 1978, it has been accepted that anti-dsDNA, U1-RNP, Sm, Ro, and La antibodies might on occasion penetrate living cells

(via Fc receptor), entering the nucleus (probably through their CDR3 region) and induce cell growth or apoptosis. Interestingly, after being internalized, autoantibodies can return to cells surface and become antigens for immunoglobulin deposition and T-cell activation (Yung and Chan 2008; Waldman and Madaio 2005).

Binding to Cell Surface

In order that autoantibodies bind directly to cells surfaces, antigens must be expressed in the outer membrane. This mechanism has been linked to the development of antiphospholipid, anti-ribosomal-P antigen, anti-Ro/La, and antierythrocyte antibodies. The binding of beta-2-glycoprotein-I (B2GPI) to phospholipids increases the affinity of anti-B2GPI antibodies to cell membranes, allowing them to bind several cell types. For example, anti-B2GPI/B2GPI complexes link to apolipoprotein E receptor 2 on platelets inducing thrombin activation, but can also stimulate monocytes, in vitro, enhancing expression of tissue factor and, during embryonic development, they bind to negatively charged trophoblasts' phospholipids impairing their invasive capacity and ultimately leading to miscarriage, a common feature of antiphospholipid syndrome. They can also interact with the neuronal tissue. Anti-ribosomal-P antibodies bind directly to the surface of T-lymphocytes, monocytes, hepatoma, and neuroblastoma cells. A neuronal surface P antigen was identified as a potential target for autoantibodies from neuropsychiatric lupus patients (Rekvig et al. 2012; Yung and Chan 2008; Núñez-Álvarez and Cabiedes 2011).

Cross-Reactivity with Extracellular Molecules

Numerous cross-reactions of anti-dsDNA antibody with different constituents of glomerular basement membrane, matrix, and podocytes have been described, explaining the existence of distinct histologic and clinical patterns of lupus nephritis. Moreover, the wide distribution of these antigens in the body can also explain the multisystemic damage that characterizes SLE. Among the several glomerular candidates, the alpha-actinin-4 from the podocytes cytoskeleton is a possible target for anti-DNA antibody cross-reaction. However, the phenomenon of cross-reactivity does not explain how high-affinity allows countless interactions with several epitopes, unless there is an intermediary agent, providing a high-affinity epitope to antibodies while anchors to target tissues. This link seems to be the circulating nucleosomes, released after apoptosis and binding to glomerulus surface molecules, exposing their DNA to autoantibodies, leading to the formation of specific dense deposits found in nephritic kidneys (Isenberg et al. 2007; Almqvist et al. 2011; Yung and Chan 2008; Waldman and Madaio 2005).

The Final Pathway: Complement Cascade Activation and Cytokine Production

Several of the mechanisms discussed previously culminate in the deposition and consumption of complement, which marks active lupus nephritis. Anti-dsDNA antibody and ICs stimulate TLR-9 and the Fc receptor promoting cytokine production, notably alfa-IFN, amplifying the inflammatory process.

Types of Autoantibodies in SLE

Several autoantibodies have been described in patients with SLE but, at present, only the anti-dsDNA, Sm, and antiphospholipid autoantibodies are included in the classification criteria of the American College of Rheumatology.

Antibodies to DNA

Several studies concluded that both mammalian and bacterial DNA activate the immune system and do not need to be fragmented to become immunogenic. In fact, the histone-1/nucleosome complexes, rather that free DNA, appear to be the real target for the anti-dsDNA antibodies (Pathak and Mohan 2011; Yung and Chan 2008).

Anti-dsDNA antibodies are present in approximately 2/3 of patients with lupus and in less than 0.5 % of healthy people or patients with other autoimmune diseases (Isenberg 2004). Variability of prevalence is probably related to

different assays and to the existence of three types of autoantibodies to DNA. The anti-single-stranded DNA antibody recognizes bases, nucleosides, oligonucleotides, and the ribose-phosphate backbone of denaturated DNA. The anti-double-stranded DNA antibody, the most frequent type, binds to the ribose-phosphate backbone of both single- and double-stranded DNA molecules, in their right-handed helical conformation, called B-DNA. The anti-z-DNA antibody binds to the rare left-handed helical conformation of the molecule of DNA (Z DNA).

At least three clinical methods can detect and quantify anti-dsDNA antibodies: *Crithidia luciliae* indirect immunofluorescence (CLIF), ELISAs, and radioimmunoassays (RIAs), namely, the Farr assay. The last is considered to be the gold standard due to its strong specificity for high-affinity anti-dsDNA antibodies. However, ELISAs are widely used, given their convenience, lack of the use of radioactive reagents, low cost, and good sensitivity to detect anti-dsDNA antibodies (Wandstrat et al. 2006; Heidenreich et al. 2009).

Over 80 % of the patients who have elevated levels of anti-dsDNA antibody develop clinically active disease within 5 years after their detection. Despite the lack of agreement of different assays, several authors were able to prove the relationship of anti-dsDNA levels with lupus activity. Several authors have suggested a sharp fall in anti-dsDNA antibody levels precedes a flare and might reflect its deposition in the glomeruli (Isenberg 2004).

In addition to reflecting the disease activity, treating patients according to anti-dsDNA antibody levels may prevent more flares rather than treating them only accordingly to clinical symptoms. Furthermore, there is a danger of overprescribing steroids in these patients, and anti-dsDNA and nucleosome antibodies have shown good correlation with clinical responses to B-cell depletion therapy (Isenberg et al. 2007; Tew et al. 2010).

In terms of prognosis, the presence of anti-dsDNA antibodies was recently identified as the best predictor for renal outcome in patients with new-onset lupus nephritis (Manson et al. 2009).

Antibodies Anti-nucleosomes

The complex organization of DNA offers a variety of targets to autoantibodies, notably histones, either in their individual form or grouped with DNA, forming nucleosomes. The nucleosome may well be the major antigen in SLE. Interestingly, in murine studies, the anti-nucleosome antibody appears before anti-dsDNA antibody and this autoantibody has also shown good correlation with disease activity, especially to lupus nephritis. Anti-histone antibodies may target individual histones components, notably H1, H2a, H2b, H3, and H4, but given the exposed position of H1, the anti-H1 antibody shows the best specificity, being found in up to 60 % of SLE patients. It has also been linked to particular manifestations (e.g., leukopenia and hemolytic anemia) and to drug-induced SLE caused by hydralazine and procainamide (Isenberg 2004; Yung and Chan 2008; Waldman and Madaio 2005; Manson et al. 2009).

Antibodies Anti-RNA

The RNA molecule is a target for autoantibodies that bind conformational epitopes of RNA, U1-snRNA, and tRNA. The linking of these autoantibodies to the cell replication machinery reinforces the potential of viruses to act as triggers of autoimmune disease. The frequency of anti-RNA antibodies in SLE patients ranges from 15 % to 25 %, varying according to the detection assays (Åhlin et al. 2012; Rahman and Isenberg 2008).

Antibodies Anti-extractable Nuclear Antigens

Anti-spliceosomal Antibodies: Anti-Sm, and Anti-Mo (Anti-ribonuclear Protein Antibodies)

The first autoantibody to intracellular molecules extracted in saline solution and not related to histones was identified in 1966. After finding that these molecules were nuclear antigens related to SLE, this autoantibody was named anti-Sm, (after the first two letters of the patient in whom it was found). A second nuclear antigen, a ribonucleoprotein (U-RNP), was late identified as another component of the spliceosome. The spliceosome is a protein complex ring that splices nuclear RNA into messenger RNA. Anti-Sm

antibodies recognize U1-, U2-, U4-, U5-, and U6-ribonucleoproteins, while anti-U1-RNP antibodies bind to the specific U1 ribonucleoprotein, exposed during cell apoptosis. According to some authors, the anti-U1-RNP antibody has the best sensitivity for SLE but insufficient specificity. In contrast, anti-Sm antibodies are present in around 10 % of Caucasian SLE patients and 30 % of those of Afro-Caribbean origin. A pathogenic role of anti-U1-RNP antibody in SLE remains unclear, although it is linked to myositis, esophageal dysmotility, Raynaud's phenomenon, and a photosensitivity rash. On the contrary, the anti-Sm antibody is associated with glomerular damage in some series and can fix complement in immune complexes. The prognostic value of overall anti-spliceosome antibodies remains unclear, although some authors relate anti-Sm antibody to worst prognosis of renal, vascular, and pulmonary disease (Isenberg et al. 2007; Rahman and Isenberg 2008; Greidinger et al. 2000).

Anti-Ro and Anti-La Antibodies

The targeting of exocrine glands by autoantibodies from the serum of a patient with Sjögrens syndrome led to the discovery of the Ro (SS-A) and La (SS-B) ribonucleoproteins. Ro consists of two peptides with 60 and 52 kDa that bind to cytoplasmic RNA and La is a phosphoprotein that binds to a variety of host and viral RNA. Unlike the anti-Sm antibodies, which are highly specific for SLE, anti-Ro and La autoantibodies have been reported with higher frequencies in Sjögren's syndrome and undifferentiated autoimmune rheumatic disease (UARD). The prevalence of anti-La antibody in SLE patients is less than 15 %, while anti-Ro antibody ranges from 25 % to 40 %, according to the assay used. For example, the anti-Ro 60-kDa antibody is seen mostly in SLE, while the anti-Ro 52-kDa antibody is almost exclusively found in Sjögren's syndrome and UARD. Their frequencies also depend on the detection assays used, given their susceptibility to be denaturated. Thus, ELISA and Western blot are the most efficient assays. Anti-Ro antibodies have been related to a high frequency of mucocutaneous symptoms, sicca syndrome, and photosensitivity. Intriguingly, anti-La antibodies were related to late-onset SLE and low incidence of renal complications (Isenberg et al. 2007; Rahman and Isenberg 2008; Peene et al. 2002).

A particular feature of these antibodies is their association with congenital heart block (CHB) and neonatal lupus, both prototypes of maternal-fetal autoimmune disease. These maternal autoantibodies pass through the placenta of about 1:20 mothers and bind to Ro/La antigens exposed in fetal cardiomyocytes, during apoptotic heart remodeling. They further disappear from the serum of infants at 6–8 months of age. Interestingly, although their mothers have these autoantibodies, they rarely develop SLE symptoms during or after pregnancy. These findings and the discordance of CHB in twins confirm that the pathogenic role of these autoantibodies is not understood (Isenberg et al. 2007; Zuppa et al. 2008).

Antibodies to Glycoproteins and Phospholipids

This heterogeneous family of autoantibodies recognizes several types of phospholipids, annexin V, prothrombin, protein C, and protein S, although their major target seems to be B2GPI. In 1995, the anti-B2GPI antibody was unequivocally related to thrombotic events and recurrent fetal losses. Secondary APS is mainly associated with SLE. In fact, lupus anticoagulant and anti-cardiolipin antibodies were found in patients with SLE. The pathogenic mechanisms of antiphospholipid antibodies are related to procoagulant and anticoagulant mechanisms, platelet activation, and inflammation. The association of IgG anti-cardiolipin antibodies with an increased risk of renal disease and of subendothelial deposits of anti-cardiolipin and anti-B2GPI antibodies to heart valve dysfunction and Libman-Sacks endocarditis reinforces the important linkage between antiphospholipid antibodies and cardiovascular and thromboembolic events in SLE patients (Isenberg et al. 2007; Núñez-Álvarez and Cabiedes 2011; Rahman and Isenberg 2008).

Antineuronal Antibodies

Antineuronal antibodies were first described in SLE patients in 1978, but their real role in neuropsychiatric lupus (NPSLE) remains unclear. Their overall prevalence in SLE patients is less than 10 %, increasing to 70–90 % in neuropsychiatric lupus. However, they lack specificity for SLE diagnosis, since they were reported in several neurologic diseases and can be detected using different sources of neuronal antigens, although the most promising target is the NMDA receptor, found mainly in the hippocampus and amygdala. These autoantibodies enter the cerebrospinal fluid by passive transfer through a permeabilized blood–brain barrier, or by direct intrathecal production, interfering in synaptic transmission or inducing apoptotic cell death. The mechanisms of their pathogenicity and the definitive relationship with SLE activity remain to be proven (Núñez-Álvarez and Cabiedes 2011; Rahman and Isenberg 2008; Colasanti et al. 2009).

Antibodies Related to Hemolytic Anemia and Immune Thrombocytopenia

Hematological disturbances are important manifestations of SLE, particularly, autoimmune hemolytic anemia due to autoantibody-mediated destruction of red cells, which may be present in the first years of the disease and related to thrombocytopenia (Evan's syndrome), lupus nephritis, and central nervous system manifestations. Although the prevalence of antibodies to red cells in SLE patients is found in <10 %, interestingly, these patients represent a subgroup with a fairly good prognosis, showing a good response to treatment. Up to 25 % of patients with SLE develop moderate to severe thrombocytopenia, by several mechanisms, including anti-platelet antibodies, ICs, antiphospholipid antibodies, and microangiopathy. These autoantibodies are found in less than 10 % of SLE patients, although their frequencies vary according to the detection techniques. Several membrane molecules have been suggested as potential targets, notably the glycoproteins IIb/IIIa and IV (Núñez-Álvarez and Cabiedes 2011).

The anti-dsDNA autoantibody was also correlated to thrombocytopenia, probably by a cross-reactivity phenomenon and CDR3 complementarity to glycoprotein IIIa, which was suggested to be an epitope mimicking dsDNA (Al-Shahi et al. 1997).

Antineutrophil Cytoplasmic Antibodies

Antineutrophil cytoplasmic autoantibodies (ANCAs) were first described in patients with necrotizing glomerulonephritis and have been associated mostly with the vasculitis, (e.g., polyangiitis with granulomatosis and microscopic polyangiitis). They bind to the myeloperoxidase and proteinase-3 of neutrophils contained in vessel walls, inducing their degranulation and further inflammation of the vessels. The phenomenon of vasculitis occurs in 35 % of patients with SLE, and ANCAs were frequently associated to nephritis and serositis. Higher levels of anti-myeloperoxidase antibodies seem to predict clinical relapse in SLE patients and longer active phases of the disease, reflecting long-standing inflammation. However, the role of ANCA in the pathogenesis of SLE remains uncertain since some patients with high ANCA levels do not develop SLE and most authors cannot dissociate the effects of ANCA from those of other antibodies found concomitantly in SLE patients (Sen and Isenberg 2003).

Anti-C1q Antibodies

Since the first discovery of an IgG against the circulating complement C1 complex in a patient with acquired angioedema, this antibody has been found in some patients with SLE and correlated with the duration and activity of this disease, especially in pediatric patients. There is also a strong correlation of the anti-C1q autoantibody levels to the activity of class III/IV lupus nephritis. Rising levels may predict renal flares.

Autoantibodies in Clinical Practice

Autoantibodies represent the hallmark of SLE and their use in clinical routine is of most importance in diagnosis, disease monitoring, and response to treatment evaluation.

The current revised ACR classification criteria include four autoantibodies: antinuclear, anti-Sm, anti-dsDNA, and antiphospholipid antibodies. All tests for anti-dsDNA antibody (*Crithidia luciliae*, BINDAZYME, FARRZYME) have moderate sensitivity and generally good specificity for SLE diagnosis, and are often best correlated with lupus nephritis. Other autoantibodies, notably anti-C1q and anti-nucleosome antibodies, are also linked to renal disease. In contrast, anti-Sm antibodies, though useful diagnostically, are not linked to renal disease (Isenberg et al. 2007; Heidenreich et al. 2009).

ANAs are a useful guide to the possibility of lupus in appropriate settings. For example, in a young woman presenting with polyarthritis and rash, whose rheumatoid factor is negative, the finding of positive ANA is likely to facilitate a lupus diagnosis. However, ANA positivity has been found after many infectious diseases and (usually in low titers) in some healthy people. Thirty to forty percent of lupus patients have antiphospholipid antibodies, but only 10–15 % have the clinical features (blood clots and miscarriages) usually associated with these antibodies (Isenberg et al. 2007; Núñez-Álvarez and Cabiedes 2011).

Other lupus antibodies may offer a guide to likely clinical features. Anti-RNPs are often linked to more overlap features (e.g., Raynaud's, myositis): anti-Ro to photosensitivity and neonatal lupus and anti-La to concomitant Sjögren's syndrome (Isenberg et al. 2007; Wandstrat et al. 2006; Peene et al. 2002; Zuppa et al. 2008).

The frequency of monitoring is also important. In terms of diagnostic confirmation, some autoantibodies are relatively stable over time, so it is not cost-effective to recheck them. Probably, around 15 % of SLE patients lose ANA positivity after diagnosis. Increasing titers of anti-dsDNA antibody accompanying a complement fall may predict exacerbations of renal and other disease manifestations. Measuring these antibodies is thus helpful in monitoring disease activity. However, there is a group of patients with persistently very high levels of anti-dsDNA antibody who remain clinically well, suggesting that not only are the levels important but also the isotype and subclass of immunoglobulin are essential to induce damage.

In conclusion, lupus patients have been associated with a wide variety of autoantibodies, though few are present in >30 % of these patients. Some are helpful diagnostically, while others point to likely clinical features. However, the precise mechanisms determining which antibodies will be truly pathogenic remain to be determined.

Cross-References

- ▶ Antiphospholipid Syndrome, Clinical Manifestations
- ▶ Autoimmune Hepatitis
- ▶ Nephritogenic Antibodies in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Systemic Lupus Erythematosus, Congenital Heart Block and Neonatal Lupus
- ▶ Systemic Lupus Erythematosus, Gender and Hormone Influences
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- ▶ Vasculitis and the Kidney

References

Åhlin E, Mathsson E, Eloranta ML, Jonsdottir T, Gunnarsson I, Rönnblom L, et al. Autoantibodies associated with RNA are more enriched than anti-dsDNA antibodies in circulating immune complexes in SLE. Lupus. 2012;21:586.

Almqvist N, Winkler TH, Mårtensson IL. Autoantibodies: focus on anti-DNA antibodies. Self/Nonself. 2011;2:11–8.

Al-Shahi R, Mason JC, Rao R, Hurd C, Thompson EM, Haskard DO, et al. Systemic lupus erythematosus, thrombocytopenia, microangiopathic haemolytic anaemia and anti-CD25 antibodies. Br J Rheumatol. 1997;36:794–8.

Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003;349:1526–33.

Colasanti C, Delunardo F, Margutti P, Vacirca D, Piro E, Siracusano A, et al. Autoantibodies involved in neuropsychiatric manifestations associated with systemic lupus erythematosus. J Neuroimmunol. 2009;212:3–9.
Ehrenstein MR, Katz DR, Griffiths MH, Papadaki L, Winkler TH, Kalden JR, et al. Human IgG anti-DNA antibodies deposit in kidneys and induce proteinuria in SCID mice. Kidney Int. 1995;48:705–11.
Greidinger EL, Casciola-Rosen L, Moris SM, Hoffman RW, Rosen A. Autoantibody recognition of distinctly modified forms of the U1-70-kDa antigen is associated with diferente clinical disease manifestations. Arthritis Rheum. 2000;43:881–8.
Heidenreich U, Mayer G, Herold M, Klotz W, Al-Jazrawi KS, Lhotta K. Sensitivity and specificity of autoantibody tests in the differential diagnosis of lupus nephritis. Lupus. 2009;18:1276–80.
Holman H. The discovery of autoantibody to deoxyribonucleic acid. Lupus. 2011;20:441.
Isenberg D. Anti-dsDNA antibodies: still a useful criterion for patients with systemic lupus erythematosus? Lupus. 2004;13:881.
Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-dsDNA antibodies: are we approaching journey's end? Rheumatology. 2007;46:1052–6.
Manson JJ, Ma A, Rogers P, Mason LJ, Berden JH, Vlag J, et al. Relationship between anti-dsDNA, anti-nucleosome and anti-alpha-actinin antibodies and markers of renal disease in patients with lupus nephritis: a prospective longitudinal study. Arthritis Res Ther. 2009;11:R154.
Muñoz LE, Janko C, Schulze C, Schorn C, Sarter K, Schett G, et al. Autoimmunity and chronic inflammation – two clearance-related steps in the etiopathogenesis of SLE. Autoimmun Rev. 2010;10:38–42.
Núñez-Álvarez CA, Cabiedes J. Pathogenic mechanisms of the anti-phospholipid antibodies. Reumatol Clin. 2011;7:72–6.
Pathak S, Mohan C. Cellular and molecular pathogenesis of systemic lupus erythematosus: lessons from animal models. Arthritis Res Ther. 2011;13:241.
Peene I, Meheus L, Veys EM, De Keyser F. Diagnostic associations in a arge and consecutively identified population positive for anti-SSA and/or anti-SSB: the range of associated diseases differs according to the detailed serotype. Ann Rheum Dis. 2002;61:1090–4.
Pisetsky DS. Antinuclear antibodies in healthy people: the tip of autoimmunity's iceberg? Arthritis Res Ther. 2011;13:109.
Pisetsky DS, Vrabie IA. Antibodies to DNA: infection or genetics? Lupus. 2009;18:1176.
Rahman A, Isenberg DA. Systemic lupus erythematosus. N Engl J Med. 2008;358:929–39.
Rahman A, Giles I, Haley J, Isenberg DA. Systematic analysis of sequences of anti-DNA antibodies – relevance to theories of origin and pathogenicity. Lupus. 2002;11:807–23.
Rekvig OP, Putterman C, Casu C, Gao HX, Ghirardello A, Mortensen ES, et al. Autoantibodies in lupus: culprits or passive bystanders? Autoimmun Rev. 2012;11:596–603.
Sen D, Isenberg DA. Antineutrophil cytoplasmic autoantibodies in systemic lupus erythematosus. Lupus. 2003;12:651–8.
Tew GW, Rabbee N, Wolslegel K, Hsieh HJ, Monroe JG, Behrens TW, et al. Baseline autoantibody profiles predict normalization of complement and anti-dsDNA autoantibody levels following Rituximab treatment in systemic lupus erythematosus. Lupus. 2010;19:146–57.
Waldman M, Madaio MP. Pathogenic autoantibodies in lupus nephritis. Lupus. 2005;14:19–24.
Wandstrat AE, Carr-Johnson F, Branch V, Gray H, Fairhurst AM, Reimold A, et al. Autoantibody profiling to identify individuals at risk for systemic lupus erythematosus. J Autoimmun. 2006;27:153–60.
Yung S, Chan TM. Anti-DNA antibodies in the pathogenesis of lupus nephritis – the emerging mechanisms. Autoimmun Rev. 2008;7:317–21.
Zuppa AA, Fracchiolla A, Cota F, Gallini F, Savarese I, D'Andrea V, et al. Infants born to mothers with anti-SSA/Ro autoantibodies: neonatal outcome and follow-up. Clin Pediatr. 2008;47:231–6.

Systemic Lupus Erythematosus, Clinical Features and Diagnosis

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Synonyms

Lupus

Definition of SLE

SLE (systemic lupus erythematosus) is a complex, unpredictable waxing and waning autoimmune disease. It is characterized by unchecked immune responses that cannot be explained by known or treatable infection. Chronic and recurrent flares of disease can potentially damage many vital organs in the body

leading to significant impact on quality of life, morbidity and mortality (Renau and Isenberg 2012; Flower et al. 2012; Alarcón et al. 1999; Jakes et al. 2012).

Clinical Manifestations

The commonest features of lupus (found at some time in most patients with SLE) are polyarticular inflammatory arthritis and certain characteristic skin rashes. The arthritis has classic rheumatic features such as morning stiffness, bilateral symmetric pattern, and involvement of proximal hand and finger joints. As such, it may resemble rheumatoid arthritis, although it tends to be non-erosive. Nevertheless, severe ligament involvement (Jaccoud's arthropathy) can occur, leading to disabling dysfunction of the joints. There are a number of inflammatory rashes ranging from a relatively mild malar rash (acute cutaneous lupus, which can sometimes occur as a prodrome to systemic flares) to an exquisitely photosensitive and sometimes widespread pruritic rash (subacute cutaneous) and to deep, painful, and disfiguring chronic manifestations, including severely scarring discoid rashes. The classification criteria include many of the other clinical features of lupus, including other mucocutaneous manifestations, seizures and psychosis, serositis, renal involvement, hematologic and serologic abnormalities (Tan et al. 1982; American College of Rheumatology website 1997; Petri et al. 2012). However, patients can develop disease in almost any organ. Raynaud's syndrome, peripheral and central neuropathies, vasculitis, myositis, carditis, pneumonitis, autoimmune hepatitis, and cerebritis can all occur without warning.

The set of classification criteria for SLE most commonly used to define patients for recent clinical trials is the 1997 update of the 1982 American College of Rheumatology Revised Criteria for Classification of Systemic Lupus Erythematosus) (American College of Rheumatology website 1997). These criteria have recently been reevaluated by the Systemic Lupus International Collaborating Clinics against a modified system (Petri et al. 2012). Both are almost equally valid in describing a population that expert physicians would agree have SLE. The major changes in the newer SLICC 2012 criteria are improvements of logic. For example, it is less easy to be falsely classified with systemic disease when there are only a few focal skin criteria using the 2012 criteria. On the other hand, it is easier to receive a systemic classification with a more localized manifestation that puts a vital organ at risk such as when there is documented lupus renal involvement.

Although the term SLE has evolved to connote widespread bodily involvement, confusion has arisen by misguided attempts to separate primary lupus syndromes and overlap syndromes from SLE as if they represent non-intersecting diseases. Several serious and even life-threatening primary lupus disorders such as discoid lupus, the antiphospholipid syndrome, or acquired, autoimmune microangiopathy may occur without any other features of lupus, and in these patients SLE classification criteria would not be met. However, these disorders cannot be distinguished, either by pathology or clinical features, from manifestations of SLE. Patients with lupus may also develop, in addition to classic lupus pathology, clinical problems traditionally assigned to other diseases such as polymyositis or Sjogren's syndrome. Although such patients are often categorized as having overlap syndromes, many are assigned more than one diagnosis, without evidence that these individuals really experience two, unrelated forms of autoimmunity.

Why is this important? Treatment approvals by regulatory agencies are increasingly restricted to the types of patients in whom clinical trials are performed, such as recently occurred with the first lupus treatment approved in more than 50 years, belimumab (Medscape Summary 2011). Patients with certain overlap syndromes and/or primary lupus syndromes are frequently excluded from trials for "SLE" and may in turn be denied access to new treatments that might have helped them. The simpler and yet more comprehensive term, lupus, therefore may apply better to all meaningful patients: those who meet classification criteria for SLE, those with moderate to

severe primary lupus syndromes, and those with lupus overlap syndromes that most likely arise from one, integrated pathophysiologic process in that given individual. All of these patients have serious illness which might benefit from the targeted agents being developed toward ameliorating lupus pathology.

Costenbader et al. (2002) proposed the Boston weighted criteria for SLE to allow a more inclusive classification to include true lupus patients who may not meet the ACR criteria. This may be an important step in the right direction. These criteria have been used in several studies since then, most importantly in the large CDC epidemiologic effort that is currently underway, through which it may be compared to both the 1982 and SLICC classification schemes (Lim et al. 2009).

The term "lupus," however, should not be used to describe people who have autoantibodies without clinical autoimmune illness (which frequently occur transiently in infections or with no associated illness in older people), nor should the term "lupus" apply to those with the disorder known as fibromyalgia. There is no evidence that fibromyalgia shares pathology with lupus, but this has sometimes caused a great deal of confusion, since many lupus patients experience symptoms similar to fibromyalgia such as depression, fatigue, and body aches, despite the fact that there are various (and multifactorial) causes for such symptoms. The law of parsimony suggests that when these symptoms occur in a lupus patient, it is probably not fibromyalgia and when they occur in a fibromyalgia patient, it is definitely not lupus.

Diagnosis Based on Pathogenesis: Science Is Entering the Clinic

Lupus has long been considered a prototype disease of multisystem autoimmunity. However, autoimmunity is a nearly outdated term today, implying a feral immune system which is storming the individual it is supposed to serve. Nothing can be less therapeutic to a patient than the often heard message they receive that their body is "attacking itself." However, it is far more logical to assume that all immune responses are defensive, not aggressive, since they do occur inside the body of the host. Damage to organs then can be looked at as a collateral event, caused by a system that is out of balance, not rogue. In fact, the growing understanding of immunity supports this model of disease better than the self-flagellating version (Elkon and Casali 2008; Shlomchik et al. 2001).

The immune system is a complex army of interacting pieces with multiple feedback loops capable of dialing up or dialing down the movement of troops to ensure a flexible response to external threats (such as microorganisms and toxins) and internal threats (such as neoplasms and bodily debris). All immune responses are capable of doing some damage to the host, but this is normally kept in reasonable check by appropriate upregulation of immune suppressive elements and vascular sanitation systems which eliminate those triggers that might perpetuate inflammation. Lupus patients, those who can be thought to share a common diagnosis, appear to share a syndrome in which there may be persistent loops of immune triggering events coupled to inadequate immune down-modulation and incomplete elimination of the triggering elements.

This feature is shared by patients with SLE and with primary organ limited lupus such as primary cutaneous lupus, autoimmune hemolytic anemia, immune thrombocytopenia, autoimmune TMHA syndrome (microangiopathy), and the antiphospholipid syndrome (Asherson et al. 2008). Overlapping types of immunologic imbalance can also be found in diseases that are currently thought of as being "not lupus" (Theander and Jacobsson 2008; Finke et al. 2009; Rai and Wakeland 2011). Notable examples include Sjogren's syndrome, polymyositis, and autoimmune thyroiditis, all of which have manifestations that can occur, indistinguishable from their primary forms, in patients who meet the various classification criteria for SLE. Similar immune imbalance is not shared, however, by most people who present with an isolated positive ANA, osteoarthritis, depression, fibromyalgia, or chronic fatigue, all of whom

have some risk of being misdiagnosed with lupus. Thus, a case can be made, based on pathogenesis, that lupus, a disease that was originally defined at a time when very little was known about the underlying immune disorder, might be both underdiagnosed and overdiagnosed even by the best clinical standards.

In a circular fashion, the following are some of the elements that seem to play a role in significant subsets of lupus patients (Elkon and Casali 2008; Shlomchik et al. 2001; Pathak and Mohan 2011)

1. Activation of the innate immune system which may have a role in the increased interferon alpha signals seen in most children and about half of all adult patients with lupus.
2. Activation of specialized cells (especially but not limited to plasmacytoid dendritic cells) which make interferon alpha.
3. Stimulation of interferon alpha inducible genes, some of which might be genetic variants which are caused by or lead to increased lupus activity. The signaling pathways thus triggered then set off a cascade of inflammatory events.
4. Cell debris caused by vascular or organ inflammation is not disposed of quickly enough by a defective reticuloendothelial system. This leads to perpetuation of the interferon alpha cycle as ribonucleotides stimulate Toll-Like Receptors, leading to further signals to accentuate the interferon alpha pathways.
5. Autoantibodies against intracellular structures, which may play beneficial roles in a measured immune response, are increased, depositing in tissues and activating complement.
6. During this process, adaptive immune responses ramp up, activating both B cells, which play multiple roles in inflammation along with the stimulation of even more autoantibody production.
7. T cells, which tend to evolve in an asymmetric fashion leading to increased TH17 cells and decreased T suppressor cells.

Both B cells and T cells have complex interactions with myeloid cells (neutrophils, monocytes, macrophages) and myeloid-like cells in the skin, kidneys, and other organs, which add to inflammatory processes in the vasculature and solid organs, particularly the skin and kidneys. At the same time, these cells seem to underperform in their functions at cleaning up debris from dead and dying cells which then litter the vasculature, leading to perpetuation of the immune response. The combination of a relative weakness in immune-dampening and immune clearance elements coupled with ongoing triggers for inflammation favors the type of immune imbalance that characterizes a lupus flare.

Optimal Diagnosis Combines Clinical Features with Strategic Diagnostic Testing

Given the complexity of the disease and the unique ways in which it might manifest in different people, the diagnosis of lupus should only be made by an experienced rheumatologist based on a combination of clinical history, clinical findings, physical examination and blood tests, and/or additional diagnostic procedures. Diagnostic testing is evolving now, but remains limited, necessitating good clinical pattern recognition at the present time.

People with mild autoimmune syndromes are sometimes told they have SLE because a weak or circumstantial history may have been coupled to a positive ANA test which in and of itself does not signify any illness. Conversely, people with significant lupus may go many years without a diagnosis because no one has thought of this explanation for their unpredictable and sometimes sequential symptoms. Classification criteria are useful in understanding the range of features that may be most common in this disease, but they need to be distinguished from strict diagnostic criteria. A skilled rheumatologist may diagnose a primary or systemic lupus without strict adherence to the Classification Criteria and similarly may determine that a patient with minor manifestations of an adequate array of such features to meet the classification criteria does not have clinically significant lupus and does not warrant the diagnosis.

For many patients, a careful clinician might suspect lupus for some time before arriving at a definitive diagnosis. Although this can be frustrating to a patient who understandably wants a clear answer and definitive solution to what might be wrong, this is often the best way to ensure a person is properly monitored and treated without risking a lifelong misdiagnosis.

The Most Important Element of Diagnosis: Prognosis

What is in a name? Once a diagnosis is given, it is important for the clinician and the patient to understand that the severity of disease on a given day, in a given year, and over a lifetime will widely vary from person to person and may also change in an unpredictable manner in a single patient. This uncertainty embedded in the diagnosis can be quite frightening, so an important part of the diagnostic process (and in the communication of this process to the patient), is to generate a reasonable prediction of expectations and formulate a meaningful rationale for treatment choices.

Of course there is no crystal ball to predict for any individual with lupus what the final outcome of their disease will be. Race, lifestyle, current blood test results, and history may be identical today for two patients, one of whom will always have mild disease and the other who will develop severe disease. Nevertheless some modeling of demographic, serologic, and clinical risk for major flares, organ damage, and overall poor prognosis (if untreated) can be performed based on certain clinical features (Renau and Isenberg 2012; Flower et al. 2012; Alarcón et al. 1999; Jakes et al. 2012). For example, Ro (SSA) and La (SSB) antibodies, which are classic features of Sjogren's Syndrome, are associated with risk for subacute cutaneous lupus, and neonatal lupus syndromes (Buyon and Clancy 2003). Antibodies to dsDNA are associated with risk for renal disease and other severe lupus manifestations (Merrill and Buyon 2005). Anticardiolipin (or related prothrombotic antibodies) confer increased risk for thrombotic manifestations (Asherson et al. 2008). Younger patients and those of Hispanic, African, or Asian background may be at risk for more severe disease (Alarcón et al. 1999; Jakes et al. 2012).

However, none of these risks are 100 % and the absence of these features does not eliminate risk. Therefore, rather than using this prognostic knowledge to falsely reassure or upset people, this kind of profiling might suggest directions for monitoring and therapy and/or suggest that more aggressive treatments will be considered earlier for some patients and provide a rationale for giving milder treatments and/or simply monitoring others.

Given strong evidence for increased risk for atherosclerosis and accelerated atherosclerosis in patients with lupus (Kao et al. 2003), diagnostic attention to all conventional risk factors for heart disease and stroke is a cornerstone of treatment, and it may be best to ensure that patients are not undertreated for the inflammatory process. This should be the same for patients with mild, moderate, or severe disease, since chronic, even low-grade inflammation may play a major role in this major cause of long-term morbidity and mortality.

Conclusion

The concept of multisystem autoimmunity which underlies the name of systemic lupus erythematosus can be confusing and sometimes misleading. There are in fact a range of clinically significant lupus syndromes all of which are sometimes linked to multiple organ disease, but many of which can also stand alone as a *forme fruste* syndrome, without additional organ involvement. Other diseases that are not considered to fall within the range of SLE or lupus have major overlapping pathophysiology and clinical features and may even occur in patients with lupus.

The danger in restricting clinical trials to those patients who meet classification criteria for SLE is that treatments might be withheld from significant subsets of people who need them. It is also problematic that some people who are quite

unlikely to benefit from new medications developed for lupus, such as those with fibromyalgia or chronic fatigue, are sometimes misdiagnosed with lupus, indeed they sometimes legitimately meet the classification criteria by a technicality without having significant inflammatory pathology. For this reason, the diagnosis and care of patients with lupus should be in the hands of a clinician with significant training and experience in this disease.

An understanding of the pathophysiology of autoimmune diseases may help to inform diagnosis of lupus. Also intrinsic to the diagnostic process is an appreciation for certain imperfect, but nevertheless helpful prognostic features of the disease, since this may help to plan the best approaches to monitoring and treatment for individual patients. Diagnostic attention to all potential risk factors for atherosclerosis, a major cause of morbidity and mortality in this population, is equally important.

Cross-References

► Systemic Lupus Erythematosus, Clinical Trials

References

Alarcón GS, Friedman AW, Straaton KV, Moulds JM, Lisse J, Bastian HM, McGwin Jr G, Bartolucci AA, Roseman JM, Reveille JD. Systemic lupus erythematosus in three ethnic groups: III A comparison of characteristics early in the natural history of the LUMINA cohort. LUpus in MInority populations: Nature vs. Nurture. Lupus. 1999;8:197–209.

American College of Rheumatology website. http://www.rheumatology.org/practice/clinical/classification/SLE/1997_update_of_the_1982_acr_revised_criteria_for_classification_of_sle.pdf. Accessed 2 Aug 2012.

Asherson RA, Cervera R, Merrill JT, Erkan D. Antiphospholipid antibodies and the antiphospholipid syndrome: clinical significance and treatment. Semin Thromb Hemost. 2008;34:256–66.

Buyon JP, Clancy RM. Neonatal lupus: review of proposed pathogenesis and clinical data from the US-based Research Registry for Neonatal Lupus. Autoimmunity. 2003;36:41–50.

Costenbader KH, Karlson EW, Mandl LA. Defining lupus cases for clinical studies: the Boston weighted criteria for the classification of systemic lupus erythematosus. J Rheumatol. 2002;29:2545–50.

Elkon K, Casali P. Nature and functions of autoantibodies. Nat Clin Pract Rheumatol. 2008;4:491–8.

Finke D, Eloranta ML, Rönnblom L. Endogenous type I interferon inducers in autoimmune diseases. Autoimmunity. 2009;42:349–52.

Flower C, Hennis AJ, Hambleton IR, Nicholson GD, Liang MH. Barbados National Lupus Registry Group Systemic lupus erythematosus in an Afro-Caribbean population: incidence, clinical manifestations and survival in the Barbados national lupus registry. Arthritis Care Res (Hoboken). 2012;64:1151–8.

Jakes RW, Bae SC, Louthrenoo W, Mok CC, Navarra SV, Kwon N. Systematic review of the epidemiology of systemic lupus erythematosus in the Asia-Pacific region: prevalence, incidence, clinical features, and mortality. Arthritis Care Res (Hoboken). 2012;64:159–68.

Kao AH, Sabatine JM, Manzi S. Update on vascular disease in systemic lupus erythematosus. Curr Opin Rheumatol. 2003;15:519–27.

Lim S, Drenkard C, Mccune WJ Helmick CG, Gordon PC, Dequire P, Bayakly R, Somers E. Population-based lupus registries: advancing our epidemiologic understanding arthritis & rheumatism. Arthritis Care Res. 2009;61:1462–6.

Medscape Summary. Belimumab indications, adverse events, and usage 2011. http://reference.medscape.com/drug/benlysta-belimumab-999632. Accessed August 2 2012.

Merrill JT, Buyon JP. The role of biomarkers in the assessment of lupus. Best Pract Res Clin Rheumatol. 2005;19:709–26.

Pathak S, Mohan C. Arthritis Res Ther. 2011;13:241–9.

Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, Bruce IN, Isenberg D, Wallace DJ, Nived O, Sturfelt G, Ramsey-Goldman R, Bae SC, Hanly JG, Sanchez-Guerrero J, Clarke A, Aranow C, Manzi S, Urowitz M, Gladman D, Kalunian K, Costner M, Werth VP, Zoma A, Bernatsky S, Ruiz-Irastorza G, Khamashta MA, Jacobsen S, Buyon JP, Maddison P, Dooley MA, Vollenhoven RF, Ginzler E, Stoll T, Peschken C, Jorizzo JL, Callen JP, Lim SS, Fessler BJ, Inanc M, Kamen DL, Rahman A, Steinsson K, Franks Jr AG, Sigler L, Hameed S, Fang H, Pham N, Brey R, Weisman MH, McGwin Jr G, Magder LS. Derivation and validation of systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012;64:2677–86.

Rai E, Wakeland EK. Genetic predisposition to autoimmunity–what have we learned? Semin Immunol. 2011;23:67–83.

Renau AI, Isenberg DA. Male vs female lupus: a comparison of ethnicity, clinical features, serology and outcome over a 30 year period. Lupus. 2012;21:781–3.

Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol. 2001;1:147–53.

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1982;25:1271–7.

Theander E, Jacobsson LT. Relationship of Sjögren's syndrome to other connective tissue and autoimmune disorders. Rheum Dis Clin North Am. 2008;34(4):935–47.

Systemic Lupus Erythematosus, Clinical Trials

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Synonyms

Lupus

Lupus: Few Approved Treatments, Disappointing Trial Results

As discussed in the entry entitled "▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis," there are currently few FDA-approved treatments for lupus. There are exactly four. These are antimalarials, corticosteroids, aspirin, and, after a 50-year lag period, the monoclonal anti-BlyS antibody, belimumab, was approved in April 2011. Given the paucity of effective and affordable approved treatments, the standard of care for most patients remains treatment with off-label medications, many of which are supported only by rudimentary anecdotal literature (Dooley and Ginzler 2006). This situation might have rapidly resolved in the last 15 years, given the number of agents entering protocolized registrational development programs specifically for lupus. But this has not happened yet.

Even for belimumab, now approved by the FDA and other worldwide regulators, the differences between treatment and placebo groups in the pivotal Phase III trials were modest (Navarra et al. 2011; Furie et al. 2011). This should not have been surprising, regardless of how effective a treatment might be, since in these year-long studies, belimumab or placebo was added to a fluid roster of background medications, allowing significant adjustments in background treatment for substantial periods at the start of the study. It was thought at the time that this approach would provide the most ethical trial design, and address problems with high dropout rates in previous studies by allowing adequate treatment in both placebo and treatment arms for sustained, 52–76 week studies. However, given a natural ceiling on the percentage of patients likely to respond to any targeted treatment, these large Phase III trials which ensured a high "placebo" or background response were mounted under an understanding that the effect size was likely to be modest.

Therefore, regulatory approvals were given for this treatment in the context of small but scientifically persuasive differences between aggressively treated arms. Unfortunately, since then, there has been only modest progress in marketing the treatment. Community confusion over the data might be part of the problem. Additionally, resistance and road blocks have arisen from the third party payers based on the small effect size, the most notable example coming from the British National Institute for Health and Clinical Excellence (NICE) which supervises coverage for treatments and failed to approve belimumab payments for the entire UK (NICE website report 2011).

In addition to the limited success of belimumab, most of the Phase II and III trials of novel biologics for lupus have failed in clinical trials completely (Merrill 2009; Bruce 2010; Xiong and Lahita 2011).

This has left many questions unanswered. Is belimumab limited in efficacy and are all of these other treatments completely ineffective for lupus? Were the esoteric outcome measures being used in this complex disease too problematic to interpret? Were there potentially illuminating subsets of these heterogeneous patients that have not to date been well enough characterized? Was the ubiquitous use of a potpourri of

background medications in these many trials interfering with the assessment of the drugs under study? Although some exploratory analysis of failed trials suggests that any of these hypotheses might be true (Merrill 2009; Bruce 2010), the definitive answer is simply not known. The community is left in the aftermath of so many disappointing trials with at best (in the case of belimumab) modest and at worst (in the case of several other theoretically promising biologics, no evidence that these treatments work. On the other hand, the evidence that these agents do not work seems equally unsubstantiated, as will be discussed below.

The First Wave of Trials

The first modern wave of registrational multicenter clinical trials for SLE to study products aimed for the market were begun in the mid- to late 1990s. These included studies of the weak androgen dehydroepiandrosterone (DHEA) (Genelabs), an antagonist of CD40 Ligand, IDEC 141 (IDEC, later to become Biogen-IDEC) and multiple trials of the anti-dsDNA antagonist Riquent (La Jolla Pharmaceutical Company), all of which failed to meet their study endpoints (Petri et al. 2002; Kalunian et al. 2002; Cardiel et al. 2008).

The Phase II program DHEA was initially designed as a steroid withdrawal study, allowing patients with mild or no disease activity to undergo a taper supplemented by study medication or placebo. Steroid tapering was found to be quite successful in both the treatment and placebo groups, although some benefits were later found for those patients with at least mild disease activity (SLEDAI greater than two) at entry. Potential modest benefits of DHEA for bone mineral density were observed, but with overall disappointing results in a population that may not have been very ill to begin with, the development program was ended.

Although Biogen and IDEC later merged, they were both developing antagonists of CD40 Ligand in the 1990s. The Biogen program was halted prematurely but the IDEC study was completed, finding virtually no difference in any measurement between treatment and placebo (Kalunian et al. 2002). Since there was a substantial improvement in the placebo group, it was speculated that perhaps, the patients were not really sick enough to determine differences in this trial, reflecting the outcomes seen in the DHEA trials.

Riquent was an oligonucleotide construct on a polyethylene platform which targeted antibodies to anti-dsDNA. It was originally designed as a "toleragen," aimed at cross-linking surface antibodies on B cells, with the aim of selectively killing only the B cells that trigger production of harmful autoantibodies. However, the treatment also seemed to bind the far more prevalent epitopes on circulating anti-dsDNA antibodies, and it is likely these became, for all practical purposes, its real target. Could a significant decrease in anti-dsDNA antibodies prevent a new flare of renal disease?

The Riquent trials which began in the 1990s but lasted well into the next decade were primarily based on a flare design. Patients who were well at baseline, but with a history of renal disease and measurable anti-dsDNA antibodies, were randomized to treatment vs. placebo and tracked for renal and other types of flares. There was no difference between treatment and placebo in these studies (Cardiel et al. 2008) and eventually the treatment was dropped.

Although there was little to no evidence that any of these therapies had clinically significant efficacy, one important cautionary generalization emerged from these early studies, underscoring thinking in the design of future trials: If patients were not significantly ill to start with, it might be difficult to assess efficacy of a treatment. Therefore, in the next wave of trials, the primary effort was directed at including only patients with significant baseline disease activity.

The Second Wave of Trials

Both active nephritis patients and significantly ill non-nephritis patients were considered appropriate patients for the second wave of trial designs,

but in each case, it seemed clear that significant background medications would need to be given to ensure the safety of these patients. Therefore, it can be generalized that the "not so sick" patients of the first wave were now going to be replaced by more acutely ill and more aggressively treated patients. The results were the same. Most trials designed in this way found no differences between treatment and placebo in primary or secondary endpoints (Merrill 2009; Bruce 2010).

As has been widely discussed, the liberal background treatments used in these trials, especially some protocols containing mandated high-dose steroids, may have been excessive and at the very least were virtually guaranteed to narrow the gap between even a highly effective treatment and placebo. Two programs with more success were the Phase III belimumab studies as discussed above (van Vollenhoven et al. 2012) and the Phase II epratuzumab study (Wallace et al. 2010). In both of these cases, there were less mandated aggressive steroids than in some of the other studies. Additionally, some evidence supported the hypothesis that a greater effect size could be distinguished when either the sickest subsets of patients or those with less aggressive background therapy were analyzed separately (11,14,18). Thus, the overall narrow ratio of illness to treatment that plagued the first wave of trials may not have been completely solved in the second wave of trials, since overall, both the illness factor and the treatment factor were increased together.

The Next Wave: Can Background Treatments Be Decreased?

One possibility similar to the design that seemed to work well in the epratuzumab Phase IIb trial is to leave patients on their background medications at entry (with or without some limited optional steroids at entry) but without allowing an increase in immune suppressants, and then to have a primary outcome visit at a relatively early time point (as soon as the treatment agent is thought to work). Once the primary endpoint date is reached, patients who have not done well could then be treated for ongoing disease with increased medications and followed for the full year for safety. Sustainability of response could also be monitored in those who do not require additional treatments.

An alternative approach is to give steroids to the patients at entry, but immediately withdraw the background immune suppressants. Steroid tapering would be aimed at the expected time that it should take the test treatment to work. As soon as a patient has a significant flare, immune suppressants can be restarted, but since the subject will be considered a permanent nonresponder at that point, this cannot confound the primary outcome measure. This design has been tried in two small pilot studies and at least one Phase II trial with results forthcoming in the near future (clinicaltrials.gov listing a; clinicaltrials.gov listing b; clinicaltrials.gov listing c).

The latter design would obviously be inappropriate for patients with active nephritis or any severe organ-threatening disease, even with close monitoring, but could be appropriate for clinically stable patients with significant arthritis and/or rash. From an ethical point of view, there are pros and cons to designs in which background medications are withdrawn and to those in which they are left in place. The greatest risk in withdrawing immune suppressants seems likely to be lupus flares. However, if the patients are entered who are not responding to the medication they are taking at baseline, if enough steroid is given to improve their condition, and if it is not withdrawn too quickly or until the test treatment would be expected to work, there is only a minimal time during which an induced flare would be allowed to continue.

Compare this to a design in which a patient must stay on ineffective therapy for a year with minimal rescue options and the withdrawal study would be a far more comfortable option for the patients. Additionally, the benefit of the withdrawal option, besides eliminating the confounding biologic effects of disparate medications, might be fewer additive infection risks. This should not be underestimated since

unacceptable infection levels have caused the termination of trials for at least two biologics that were added to aggressive background immune suppression (pharmastrategy blog 2010; Ginzler et al. 2012).

Biology May Be Destiny in Lupus Trials

Given the known biologic and clinical heterogeneity of lupus patients (as discussed in the entry "▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis"), it is unlikely that any given targeted treatment will work for all patients with lupus or in any given patient under all circumstances, especially with the potentially confounding impact of background therapies. In any unselected group of patients, this puts a ceiling on potential efficacy. But does this mean that biologic subsets more likely to respond might be identified? Without hoping that any approach to select among these complex patients is likely to be 100 % effective, it seems likely that subsets who are more likely to respond to given targeted treatments than an unselected lupus population could certainly be sorted out. Interestingly, belimumab may have gained its modest success only because of a biologic prescreening of patients with significant titer autoantibodies for entry into the Phase III trials who might logically be more likely to benefit from B cell targeted therapy such as this agent. The potential for various disturbances in immune equilibrium by different background treatments, any one of which could be interfering with the observable effects of a given test treatment, may become an important variable in the design of future trials.

Another important consideration is the possibility that interruption of one pathway among the complex disordered signals in SLE could initiate additional problems. The underlying biology of response and nonresponse may differ among certain subsets of patients but it would make sense to try and characterize these changes early in the development process for a new biologic in order to optimize patient selection for the later, more expensive pivotal trials.

The Measurement Is the Message

Another unresolved issue in lupus clinical trials is whether the endpoints being measured are either accurate or clinically meaningful. In trials which included aggressive background treatments and an endpoint that defined nonresponse, at least in part, by a single mild flare (one BILAG B) at any given visit, the response rates for both the treatment and placebo patients were extremely low (20–30 %) (Merrill et al. 2010a, b). However, exploratory exploration of alternative endpoints showed that overall disease activity improved in both treatment and placebo groups in these studies. Although exploratory endpoints must be interpreted with caution, this illustrates the adage that the answers received depend on the question asked.

In the belimumab and epratuzumab studies, nonresponse depended on a more significant (and likely more clinically meaningful) measurement of disease (Wallace et al. 2010; van Vollenhoven et al. 2012). Both used composite responder indices and, importantly, a landmark analysis. Unlike the previous failed trials in which a minor flare midway through the study rendered permanent nonresponse, these studies allowed those with transient interim flares to be contenders for response at the subsequent visit and, most importantly, the primary endpoint date. Although there are issues with landmark analysis, not the least of which is the consistency of responses throughout the year, this can be partially addressed with a multiple landmark analysis, as was done for both the belimumab and epratuzumab trials.

Conclusions

On the basis of data from recent clinical trials in lupus, it can be hypothesized that there have been excessive background treatments used in many studies. Given the likelihood that there is a ceiling on the percentage of unselected, heterogeneous lupus patients likely to respond to any given targeted biologic, allowing an aggressive

standard of care in the background risks a high rate of "placebo group" efficacy, which, coupled to the potential for either positive or negative interactions with the mechanism of the test drug, is likely to minimize potential differences between placebo and even an effective treatment. In revisiting the ethics of a trial design in which patients are given novel biologics in addition to close to optimal standard of care, the downsides of excessive immune suppression, including risks for infection, should also be considered.

Optimal ethics would dictate a trial design that is both optimally safe from the point of view of disease flares and infectious risk, and optimally compatible with an interpretable efficacy assessment. If immune suppressant withdrawal could be coupled to a steroid burst and taper, this would ensure patient comfort and safety as the trial begins. If patients could then be treated in a timely manner at the time of any significant flare, but nevertheless allowed to continue in the study as a designated "nonresponder," this might become an even more ethical trial design than those which enforce the continuation of treatments that are not working at baseline and withhold rescue therapy during the latter phases of the study.

With an evolving understanding of variables introduced by the heterogeneous underlying biology of lupus patients, the impact of background treatments on the pathways affected by investigational biologics, and optimal endpoints to appropriately distinguish clinically meaningful differences between an effective treatment and placebo, it can be hoped that trial designs will become more effective and interpretable in the near future.

References

Bruce I. Re-evaluation of biologic therapies in systemic lupus erythematosus. Curr Opin Rheumatol. 2010;22:273–7.

Cardiel MH, et al. Abetimus sodium for renal flare in systemic lupus erythematosus: results of a randomized, controlled phase trial. Arthritis Rheum. 2008;58:2470–80.

Clinical Trials.gov listing a. Biomarker-linked outcomes of cellcept in lupus arthritis clinicaltrials. gov identifier NCT00594932. http://clinicaltrials.gov/ct2/show/NCT00594932?term=oklahoma+medical+research+foundation&rank=1. Accessed 2 Aug 2012.

Clinicaltrials.gov listing b. Biomarkers of lupus disease: serial biomarker sampling in patients with active systemic lupus erythematosus (SLE) (BOLD) clinicaltrials.gov identifier NCT00987831. http://clinicaltrials.gov/ct2/show/NCT00987831?term =Oklahoma+Medical+Research+Foundation&rank=3. Accessed 2 Aug 2012.

Clinicaltrials.gov listing c. A study to evaluate the efficacy and safety of rontalizumab in patients with moderately to severely active systemic lupus erythematosus (ROSE) clinicaltrials.gov identifier NCT00962832. http://clinicaltrials.gov/ct2/show/NCT00962832?term=Rontalizumab&rank=1. Accessed 2 Aug 2012.

Dooley MA, Ginzler EM. Newer therapeutic approaches for systemic lupus erythematosus: immunosuppressive agents. Rheum Dis Clin North Am. 2006;32:91–102.

Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzová D, Sanchez-Guerrero J, Schwarting A, Merrill JT, Chatham WW, Stohl W, Ginzler EM, Hough DR, Zhong ZJ, Freimuth W, van Vollenhoven RF, BLISS-76 Study Group. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. Arthritis Rheum. 2011;63:3918–30.

Ginzler EM, Wax S, Rajeswaran A, Copt S, Hillson J, Ramos E, Singer NG. Atacicept in combination with MMF and corticosteroids in lupus nephritis: results of a prematurely terminated trial (abstract). Arthritis Res Ther. 2012;14:R33.

Kalunian KC, Davis Jr JC, Merrill JT, Totoritis MC, Wofsy D, IDEC-131 Lupus Study Group. Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 2002;46:3251–8.

Merrill JT. What was wrong and might now go right with clinical trials for lupus? Curr Rheumatol Rep. 2009;11:235–7.

Merrill JT, Neuwelt CM, Wallace DJ, et al. Efficacy and safety of rituximab in patients with moderately-to-severely active systemic lupus erythematosus (SLE): results from the randomized, double-blind phase II/III study EXPLORER. Arthritis Rheum. 2010a;62:222–33.

Merrill JT, Burgos-Vargas R, Westhovens R, et al. The efficacy and safety of Abatacept in patients with non-life-threatening manifestations of Systemic Lupus Erythematosus: results of a 12-month exploratory study. Arthritis Rheum. 2010b;62:3077–87.

Navarra SV, Guzmán RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, Li EK, Thomas M, Kim HY, León MG, Tanasescu C, Nasonov E, Lan JL, Pineda L, Zhong ZJ, Freimuth W, Petri MA, BLISS-52 Study Group. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus:

a randomised, placebo-controlled, phase 3 trial. Lancet. 2011;377:721–31.
NICE website report. National Institute for Health and Clinical Excellence 2011. http://www.nice.org.uk/newsroom/pressreleases/SystemicLupusBelimumabFAD.jsp. Accessed 12 Aug 2012.
Petri MA, Lahita RG, Van Vollenhoven RF, Merrill JT, Schiff M, Ginzler EM, Strand V, Kunz A, Gorelick KJ, Schwartz KE, GL601 Study Group. Effects of prasterone on corticosteroid requirements of women with systemic lupus erythematosus: a double-blind, randomized, placebo-controlled trial. Arthritis Rheum. 2002;46:1820–9.
pharmastrategy blog. http://pharmastrategyblog.com/2010/03/roche-ocrelizumab-astrazeneca-cediranib-bite-the-dust.html/. Accessed 2 Aug 2012.
van Vollenhoven RF, Petri MA, Cervera R, Roth DA, Ji BN, Kleoudis CS, Zhong ZJ, Freimuth W. Belimumab in the treatment of systemic lupus erythematosus: high disease activity predictors of response. Ann Rheum Dis. 2012;71:1343–9.
Wallace DJ, Kalunian K, Petri M,Strand V,Kilgallen B, Barry A, Gordon C. Epratuzumab demonstrates clinically meaningful improvements in patients with moderate to severe systemic lupus erythematosus (SLE): results from EMBLEM™, a phase IIb study. Ann Rheum Dis (EULAR abstract): SATO212. 2010.
Xiong W, Lahita RG. Novel treatments for systemic lupus erythematosus. Ther Adv Musculoskelet Dis. 2011;3:255–66.

Systemic Lupus Erythematosus, Congenital Heart Block and Neonatal Lupus

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Synonyms

Neonatal autoimmune disease; Neonatal lupus

Definition

Neonatal lupus is a condition affecting newborns born to mothers with systemic lupus erythematosus who have anti-Ro and anti-La antibodies. The most important clinical manifestation of neonatal lupus is congenital heart block.

Introduction

Neonatal lupus is the archetype of a group of diseases known as neonatal autoimmune diseases. The neonatal autoimmune diseases include neonatal lupus, neonatal phospholipid syndrome, neonatal Graves disease, neonatal polymyositis and dermatomyositis, neonatal Behcet's disease, and neonatal Kawasaki disease (Chang 2012). The maternal autoantibodies in neonatal lupus are directed against the ribonucleoproteins (RNP) SS-A (Ro), SS-B (La), and less commonly U1-RNP (Harley et al. 1985). In neonatal antiphospholipid syndrome, the relevant antibodies are directed against cardiolipin and β2-glycoprotein-1. In many of these other neonatal autoimmune disease, the offending autoantibody has not been determined.

Historical Background

Congenital heart block was first described in 1901 (Morquio 1901). The first reports of an association between congenital heart block and maternal systemic lupus erythematosus appeared in 1966 (Hull et al. 1966). It was not until about a decade later that autoantibodies were detected in Sjogren's syndrome and systemic lupus erythematosus (SLE). These autoantibodies were originally found by immunodiffusion and were designated the precipitins SS-A, SS-B, and rheumatoid arthritis precipitins. Two antigenic Ro polypeptides were then reported, a 60 kd and a 52 kd polypeptide. These were designated as Ro60 and Ro52. An antigenic determinant of La that is implicated in congenital heart block is a 48kd protein which shares no homology with the SS-A/Ro polypeptides. The function of SS-B/La involves a role in maturation of RNA polymerase III transcripts and in the termination of transcription, and it appears to exist in a complex with 5S rRNA and tRNA, as well as

the SS-A/Ro polypeptides. An association between maternal autoantibodies to SS-A/Ro and SS-B/La was reported in the mid-1980s (Harley et al. 1985). More recently, anti-U1-RNP antibodies have been associated with neonatal lupus.

Epidemiology/Prevalence

The incidence of congenital heart block is estimated to be about 1 in 20,000 live births. Neonatal lupus occurs in about 1–2 % of offspring of mothers with systemic lupus erythematosus and who possess anti-Ro or anti-La antibodies. If the mother has previously had a child with neonatal lupus (presenting with congenital heart block, cutaneous manifestations, or both), the incidence of disease increases 10-fold (Buyon et al. 1998). A US registry of 325 neonates born to antibody positive mothers revealed a mortality rate of 17.5 % and a 10 year survival rate of 86 %. In neonates with only isolated heart block, the fatality rate was 7.8 %. Seventy percent of neonates required pacing. Risk factors for fatality include hydrops fetalis and endocardial fibroelastosis (Izmirly et al. 2011).

Clinical Presentation

The classical presentation of neonatal lupus is congenital heart block. Other cardiac manifestations may include cardiomyopathy, fetal pericarditis and myocarditis, endocardial fibroelastosis, transient prolonged QT interval, and sinus bradycardia. Structural cardiac anomalies such as patent ductus arteriosus (Stephensen et al. 1981), ventricular septal defects, atrial septal defects, and pulmonary valve stenosis are not commonly seen in neonatal lupus but have also been reported.

Cutaneous manifestations may occur in about 25–50 % of cases. These are usually self-limiting and resolve by about 6–8 months of life, corresponding approximately to the time when maternal antibodies disappear from the neonatal circulation. The rash is generally described as erythematosus annular lesions about 1 cm in diameter or as arcuate macules with slight central atrophy and raised active margins. The physical location of the rash usually includes the scalp and periorbital areas but may also involve other parts of the body including the trunk. In some cases there is a typical "Raccoon's eye" or "Owl's eye" appearance. The rash can be exacerbated by exposure to sunlight. Histologically, the rash has been described as similar to subacute cutaneous lupus erythematosus. Alternatively, the rash can present as telangiectasias.

Other system involvement includes the hepatic system, the neurological system, and the hematologic system. Hepatic manifestations can present in three ways, with the most mild being a transient elevation of liver transaminases. The most severe form is fulminant liver failure and the third form is hepatobiliary cholestasis without or with only mild elevation of liver enzymes. The hematologic manifestations can include thrombocytopenia, anemia, or neutropenia. Macrocephaly and hydrocephalus have been reported as neurological manifestations of neonatal lupus (Silverman and Jaeggi 2010). Chondrodysplasia punctata has also been reported as a skeletal manifestation.

Diagnosis and Differential Diagnosis

Unlike SLE, criteria for the diagnosis of neonatal autoimmune disease do not exist. The diagnosis, however, is not difficult to make from a clinical standpoint. The presence of anti-Ro or anti-La antibodies in a mother of a neonate with congenital heart block is easily detectable and reflects a high likelihood of the correct diagnosis. The search for these antibodies requires a high index of suspicion. Other non-cardiac manifestations may support the diagnosis, and the family history of having a previous child with neonatal lupus also helps, since the incidence of neonatal lupus increases about 10-fold in the face of a previous child with the disease.

The differential diagnosis of the rash may include urticaria, seborrheic dermatitis, erythema

annulare centrifugum, erythema multiforme, annular erythema of infancy, erythema marginatum, and tinea corporis. The presence of an immune response to the 50kd La protein as well as U1-RNP seem to confer risk of developing cutaneous symptoms, while antibodies to the classical 60kd Ro and 52kd Ro proteins are more likely associated with complete heart block (Yukiko 2000).

Other Neonatal Autoimmune Diseases

Another well-described neonatal autoimmune disease is neonatal antiphospholipid syndrome. It is a rare disease but can be associated with a significant increase in fetal and neonatal mortality. There have been very few reports of thrombosis as a result of antiphospholipid syndrome, but more commonly, morbidity is related to poor fetal growth, premature labor, preeclampsia, and thrombocytopenia (Abrahams et al. 2012). The pathophysiology of neonatal antiphospholipid syndrome is not well understood.

Pathogenesis

The role of autoantibodies in the pathogenesis of neonatal lupus is still undetermined. The search for a cross reacting cardiac antigen has revealed that L-type calcium channels may interact with the anti-Ro or anti-La antibodies and that this may impact normal cardiac conduction, leading to heart block. But this still does not explain why only a small percentage of neonates born to mothers with these autoantibodies develop congenital heart block.

The primary theory behind the pathogenesis of neonatal lupus involves the concept of apoptosis. Apoptosis is already believed to play a role in both systemic lupus erythematosus and drug-induced lupus, albeit through different pathways and with different effects (Chang and Gershwin 2011). In the case of neonatal lupus, apoptosis may lead to the migration of Ro and La antigens from the nucleus to the cell surface, thus presenting them for binding with autoantibodies from the mother. The timing of these events may be a potential reason why not all mothers with these autoantibodies bear a child with congenital heart block. However, even if an interaction between maternal autoantibodies and the corresponding antigens in the fetus or neonate occurs, that does not necessarily indicate that this is the pathogenesis of disease. One theory is that Ro and La have no other significance other than they lead to the generation of an immune complex with the corresponding maternal autoantibodies, and rather, it is the resulting inflammation in the heart tissues in proximity to these immune complexes that results in damage to structures instrumental in cardiac pacing, such as the atrioventricular node. Another possibility is that there is immunologically cross reactivity between the molecules Ro and La and critical proteins that are engaged in normal cardiac conduction, as in the case of the aforementioned L-type calcium channels (Boutjdir 2000). Interestingly, β2-glycoprotein 1 appears to confer a protective effect for the development of congenital heart block in anti-Ro and anti-La positive mothers (Reed et al. 2011).

Further studies have defined a p200 epitope of Ro52 that encompasses amino acids 200–239 as a target of autoantibodies. p200-autoantibodies have been found to be present at higher levels in mothers of children with all degrees of severity of atrioventricular block (Strandberg et al. 2008). While this may not help establish a cause and effect relationship between the autoantibodies and disease nor may it not help explain the role of Ro52 in the pathogenesis, it does have the potential to provide clinicians with a serologic marker that can help define the risk of developing congenital heart block.

The role of T cells in the development of neonatal lupus is complex. The populations of the various T cell subtypes in cord blood have been studied, and it appears that regulatory T cells are deficient, which contradicts the belief that the neonatal immune system is overall tolerogenic. In other words, reduced regulatory T cell function allows for a more immunogenic immune system. However, this may actually

reflect a window of opportunity to develop autoimmune disease, which occurs when there is a sufficiently unregulated T cell response coupled with the appearance of maternal autoantibody in the fetus and the appearance of autoantigen on the surface of apoptotic cardiac cells. It is only when these conditions are met that autoimmunity occurs. This theory is highly speculative, but it is interesting to note that Setiady et al. were able to artificially manipulate the development of autoimmune ovarian disease (AOD) in mice by transfer of adult populations of T cells either rich or deficient in CD4 + CD25+ regulatory T cells (Setiady et al. 2005).

Neonates have also been shown to have increased inhibitory receptor expression on their immune cells, probably to facilitate the development of tolerance. Examples of these inhibitory receptors are CD31, leukocyte-associated immunoglobulin (Ig)-like receptor-1 (LAIR-1), signal-regulatory protein alpha (SIRPa), CD200, and others. While there is no evidence that these are reduced in neonates who develop congenital heart block, this is a valid consideration for future investigations into the role of T cells in this disease.

Innate immunity may play a role in neonatal lupus. Pattern recognition receptors such as toll-like receptors (TLRs) are an integral part of the innate immune system, which is critical for early protection against dangerous entities, such as microbes. A role of TLRs in rheumatic diseases stems from a defective ability to distinguish between self and dangerous antigens, and nucleic acids have been found to be able to act as ligands for TLRs. In the case of neonatal lupus, it is possible that the formation of an interaction between TLRs and Ro or La leads to activation of innate immunity and the production of a not necessarily healthy response to the nuclear antigen, leading to clinical manifestations of heart block and other signs of neonatal lupus.

This role of TLR in the pathogenesis of neonatal lupus may perhaps explain why antimalarial drugs such as hydroxychloroquine may have clinical benefits, since these drugs have been shown to inhibit interferon-γ-dependent TLR-7 activation, and TLR-7 has been found to play a role in mediating inflammation in congenital heart block patients (Clancy et al. 2010a).

Genome-wide association studies (GWAS) have identified two candidate loci at the HLA region 6p21 and the non-HLA region 21q22 to be risk alleles for the development of congenital heart block (Clancy et al. 2010b). Earlier HLA studies suggest that HLA-Cw3 may be associated with neonatal lupus, but this association has not been confirmed.

Prognosis

The progression of congenital heart block is variable. In the cases of milder forms of congenital heart block, there is usually no progression to more severe forms. However, neonates who have second degree heart block or worse will usually progress to complete heart block. Over 90 % of this group of neonatal lupus patients will eventually require cardiac pacing. Management and treatment measures, as outlined below, are not successfully in reversing this trend. In cases were heart block is severe, there may be involvement of cardiac muscle, leading to cardiomyopathy and fibrosis with a corresponding significantly higher morbidity and mortality. It is not yet clear whether neonates with congenital heart block are more prone to developing autoimmune diseases later in life. It is also not known if there is an increased incidence over that which would be expected from the family history of the mother alone. Interestingly, neonates with congenital heart disease can be born to mothers without lupus who have antibodies to Ro and La, and these women almost always develop autoimmune disease subsequently. In these cases, the diagnosis of neonatal lupus may be the first indication of autoimmune disease in the mother.

A 25-year follow-up study of the long-term prognosis of congenital heart block indicates that the majority of patients are able to lead normal and productive lives and are able to carry a child to term (Capone et al. 2012). Most cases are diagnosed at birth and pacemaker management is instituted early. Clearly, if there

is associated myocardial disease that is not treated, then the morbidity and mortality increases significantly.

Management

The management of neonatal lupus is generally supportive. Agents used have included corticosteroids, intravenous immunoglobulin (IVIg), and hydroxychloroquine. Results are inconclusive. Studies using corticosteroids have not demonstrated that prenatal administration to the mother or postnatal administration can prevent the progression of heart block (Friedman et al. 2009). However, there is some indication that the morbidities of heart block, including pericarditis, myocardiopathy, pleural effusion, hydrops, and other serious complications may be prevented. One must also consider the potential adverse effects of corticosteroids on the fetus, depending on the dosage administered.

The use of intravenous immunoglobulin (IVIg) has also been extensively studied in the treatment of congenital heart block. IVIg 400 mg/kg given in the second trimester (12–24 weeks) did not reduce the incidence of 2nd or 3rd degree cardiac block (Pisoni et al. 2010).

Based on previous experience with the use of antimalarials for rheumatic diseases, hydroxychloroquine has also been studied in neonatal lupus. Early studies showed that hydroxychloroquine was able to reduce the risk of congenital heart block if it was given before 16 weeks of gestation. Furthermore, in a historical cohort study of 257 pregnancies to mothers with positive anti-SSA/Ro titers and a history of a previous child with congenital heart block, being treated with hydroxychloroquine was associated with a significantly reduced risk of recurrent congenital heart block (7.5 % recurrence rate in the hydroxychloroquine-treated group versus 21.2 % in the non-treated group) (Izmirly et al. 2010).

Mothers who are at potential risk for developing a child with neonatal lupus should be screened for autoantibodies to Ro and La. Fetal echocardiograms should be performed to determine if heart block exists and for any complications of heart block. Cardiac pacing is an important facet of the treatment of neonatal lupus, both postnatally and prenatally with invasive fetal pacing to prevent hydrops. Overall, about 60–70 % of congenital heart block patients will ultimately require cardiac pacing. This figure is higher in patients with 2nd or 3rd degree heart block.

Family counselling and planning are important aspects of the management strategy for neonatal lupus. The family should be educated on the probability of having a child with neonatal lupus and how this may impact the family. If the mother has had a history of a previous child with neonatal lupus, then she should be counselled regarding the much higher risk of having another child with congenital heart block. The parents should also be advised on how the fetus may be monitored during the prenatal period and what to expect after the child is born. Mothers with systemic lupus erythematosus can have very successful pregnancies but need to be informed of the risks with a great deal of sensitivity, empathy, and support.

Conclusion

The most important clinical manifestation of neonatal lupus is heart block. This is the main cause of death. Other clinical manifestations are usually self-limiting and do not usually result in mortality or long-term morbidity. The cardiac manifestations of neonatal lupus can be variable, ranging from first-degree heart block to complete heart block. Hydrops fetalis is a significant complication if heart block occurs in the prenatal period.

The pathogenesis of neonatal lupus is unknown. Molecular mimicry mechanisms involving autoantibodies to Ro and La may or may not play a role in pathogenesis, and other mechanisms may be involved, including apoptosis, maternal microchimerism, and genetic factors.

The management of neonatal lupus involves supportive care. Studies using corticosteroids,

intravenous gamma globulin have yielded equivocal results. The use of hydroxychloroquine appears to be more efficacious. The more severe cases of congenital heart block ultimately require cardiac pacing.

Cross-References

- Pregnancy in Systemic Lupus Erythematosus
- Sjögren's Syndrome
- Systemic Lupus Erythematosus, Autoantibodies
- Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- Systemic Lupus Erythematosus, Gender and Hormone Influences
- Systemic Lupus Erythematosus, Genetics
- Systemic Lupus Erythematosus, Pathogenesis
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Abrahams VM, Borghi MO, et al. What is the mechanism(s) of antiphospholipid antibody-mediated pregnancy morbidity? In: Erkan D, Pierangeli SS, editors. Antiphospholipid syndrome: insights and highlights from the 13th international congress on antiphospholipid antibodies. New York: Springer; 2012. p. 79–101.

Boutjdir M. Molecular and ionic basis of congenital complete heart block. Trends Cardiovasc Med. 2000;10(3):114–22.

Buyon JP, Hiebert R, et al. Autoimmune-associated congenital heart block: demographics, mortality, morbidity and recurrence rates obtained from a national neonatal lupus registry. J Am Coll Cardiol. 1998;31(7):1658–66.

Capone C, Buyon JP, et al. Cardiac manifestations of neonatal lupus: a review of autoantibody-associated congenital heart block and its impact in an adult population. Cardiol Rev. 2012;20(2):72–6.

Chang C. Neonatal autoimmune diseases. Lupus. 2012;21:1487–91.

Chang C, Gershwin ME. Drug-induced lupus erythematosus: incidence, management and prevention. Drug Saf. 2011;34(5):357–74.

Clancy RM, Alvarez D, et al. Ro60-Associated single-stranded RNA links inflammation with fetal cardiac fibrosis via ligation of TLRs: a novel pathway to autoimmune-associated heart block. J Immunol. 2010a;184(4):2148–55.

Clancy RM, Marion MC, et al. Identification of candidate loci at 6p21 and 21q22 in a genome-wide association study of cardiac manifestations of neonatal lupus. Arthritis Rheum. 2010b;62(11):3415–24.

Friedman DM, Kim MY, et al. Prospective evaluation of fetuses with autoimmune-associated congenital heart block followed in the PR interval and dexamethasone evaluation (PRIDE) study. Am J Cardiol. 2009;103(8):1102–6.

Harley JB, Kaine JL, et al. Ro (SS-a) antibody and antigen in a patient with congenital complete heart block. Arthritis Rheum. 1985;28(12):1321–5.

Hull D, Binns BA, et al. Congenital heart block and widespread fibrosis due to maternal lupus erythematosus. Arch Dis Child. 1966;41(220):688–90.

Izmirly PM, Kim MY, et al. Evaluation of the risk of anti-SSA/Ro-SSB/La antibody-associated cardiac manifestations of neonatal lupus in fetuses of mothers with systemic lupus erythematosus exposed to hydroxychloroquine. Ann Rheum Dis. 2010;69(10):1827–30.

Izmirly PM, Saxena A, et al. Maternal and fetal factors associated with mortality and morbidity in a multiracial/ethnic registry of anti-SSA/Ro-associated cardiac neonatal lupus. Circulation. 2011;124(18):1927–35.

Morquio L. Sur une maladie infantile et familale characterisee par des modifications permanentes du pouls, des attaques syncopales et epileptiformes et al. Mort subite. Arch Med Inf. 1901;4:467–75.

Pisoni CN, Brucato A, et al. Failure of intravenous immunoglobulin to prevent congenital heart block: findings of a multicenter, prospective, observational study. Arthritis Rheum. 2010;62(4):1147–52.

Reed JH, Clancy RM, et al. beta2-Glycoprotein I and protection from anti-SSA/Ro60-associated cardiac manifestations of neonatal lupus. J Immunol. 2011;187(1):520–6.

Setiady YY, Agersborg S, et al. Neonatal autoimmune disease: influence of CD4+ CD25+ regulatory T cells. Int Rev Immunol. 2005;24(3–4):227–45.

Silverman E, Jaeggi E. Non-cardiac manifestations of neonatal lupus erythematosus. Scand J Immunol. 2010;72(3):223–5.

Stephensen O, Cleland WP, et al. Congenital complete heart block and persistent ductus arteriosus associated with maternal systemic lupus erythematosus. Br Heart J. 1981;46(1):104–6.

Strandberg L, Winqvist O, et al. Antibodies to amino acid 200–239 (p200) of Ro52 as serological markers for the risk of developing congenital heart block. Clin Exp Immunol. 2008;154(1):30–7.

Yukiko N. Immune responses to SS-a 52-kDa and 60-kDa proteins and to SS-B 50-kDa protein in mothers of infants with neonatal lupus erythematosus. Br J Dermatol. 2000;142(5):908–12.

Systemic Lupus Erythematosus, Gender and Hormone Influences

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Synonyms

Estrogen; Sex; Systemic lupus erythematosus

Definition

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease which afflicts mainly women in the reproductive years and leads to significant morbidity and mortality. The disease follows an unpredictable relapse-remission type of course with variable phases of disease quiescence interrupted by sporadic flares of intense disease activity. The production and deposition of autoantibodies and inflammatory cell infiltration lead to damage of multiple target organs such as the skin, kidneys, and brain. Deregulation of both innate and adaptive arms of the immune system contributes to the disease pathogenesis. While the exact cause of SLE is not known, a combination of genetic and environmental factors (UV radiation, drugs, hormones, etc.) is thought to be responsible for the onset and severity of disease.

X Chromosome and Sex Hormones

Ninety percent of patients afflicted with SLE are women, indicating that the female gender is an important factor in disease development. The gender bias in SLE reflects the role of sex chromosomes as well as sex hormones. A skewed X chromosome-inactivation pattern, sex hormone defects, and reproductive history are factors that contribute to the breakdown of tolerance leading to autoimmunity (Selmi et al. 2012). Several X chromosome defects have been reported in patients with SLE. These include gene translocation causing triplication of genes, X duplication with an increased incidence of SLE in males with Klinefelter's (XXY) syndrome, and demethylation of genes such as CD40L on the X chromosome, resulting in overexpression of CD40L. The importance of the X chromosome was shown in a pristane-induced model of lupus wherein the XX sex chromosome complement conferred increased susceptibility to disease over the XY^{-} mice (Selmi et al. 2012).

The disease manifests predominantly in the reproductive phase such that before puberty, the female to male prevalence is 3:1, which increases to 9:1 after the onset of puberty, implicating the role of sex hormones in disease pathogenesis. Further, evidence points to an increased estrogenic environment in both men and women with SLE. Historically, animal studies using both NZB/NZW and MRL/lpr mouse models of lupus have shown that lupus-prone female mice succumb to disease sooner than male mice. Early studies performing gonad removal (ovaries or testicles) with subsequent improvement or worsening of disease suggested the important role of hormones. Female mice survived longer after ovariectomy than castrated male mice. Administration of estrogen worsened the disease, while androgen supplements improved the disease in both female or male castrated mice (Lahita 1999, 2011). While serum estrogen levels are not significantly altered in patients with SLE, androgen levels are found to be significantly lower. There appears to be an increase in estrogen metabolism in SLE. Increased levels of the feminizing 16-hydroxyestrone and estriol metabolites occur in the serum of patients resulting from an increased oxidation of the androgen precursor dehydroepiandrosterone (DHEA). On the other hand, androgen levels specifically DHEA are low in lupus patients. Besides estrogen, the female hormone prolactin is associated with worse renal disease in lupus-prone mice, while the prolactin inhibitor bromocriptine improved disease and prolonged survival in these mice (Lahita 1999).

Estrogen and Immune Responses

Estrogen is known to regulate the immune system by modulating cytokine production. Cytokine abnormalities are an important component in the aberrant immune response in patients with SLE. The immune response in SLE is characterized by a switch from a T helper (Th) 1 to a Th2 type of cytokine environment such that cytokines interleukin (IL)-4, IL-6, and IL-10 are increased while IL-2 and IFN-γ are decreased in serum from patients. High doses of estrogen are known to promote Th2 cytokine (IL-4, IL-10, TGFβ) production. In contrast, estrogen suppresses the Th1 (IL-12, TNF-α, IFN-γ) cytokines. In the lupus-prone NZB/NZW mice, high serum estrogen levels correlated with low IL-2 levels. Furthermore, estrogen treatment increased TNF-α and IL-6 levels after lipopolysaccharide (LPS) challenge in both normal and lupus-prone MRL/lpr mice, and these effects were reversed by the selective estrogen receptor modulator tamoxifen. Animal studies have shown that mice treated with synthetic estrogen were susceptible to *L. monocytogenes* bacterial infection and their splenocytes produced less IL-2, while increased IL-17 production was seen in splenocytes from estrogen-treated mice (Salem 2004; Kassi and Moutsatsou 2010).

While hormones especially estrogens are important contributors in the expression of disease, their exact molecular role and mechanisms of action are still poorly understood. Some of the first direct molecular evidence into the role of estrogen in autoimmunity came from studies performed in non-autoimmune mice transgenic for the heavy chain of a pathogenic anti-dsDNA antibody. Estrogen upregulates expression of the antiapoptotic molecule Bcl-2 and promotes survival of autoreactive B cells, allowing their escape from tolerance induction (Cohen-Solal et al. 2008). Furthermore, estrogen enables the survival and persistence of autoreactive T cells by downregulating FasL and suppressing activation-induced cell death of human SLE T cells (Lang 2004). Studies performed in human peripheral blood T cells have shown that estrogen increases the expression of calcineurin mRNA and the encoded protein phosphatase (PP) 2B activity in an estrogen receptor-dependent manner. PP2B induces dephosphorylation of the nuclear factor of activated T-cell (NFAT) transcription factor and subsequent nuclear translocation and binding to target genes such as CD40L. Estrogen may contribute to the increased T-cell cognate help to autoreactive B cells, as estradiol administration was shown to upregulate the expression of CD40L in T cells from lupus patients but not healthy individuals (Rider and Abdou 2001). Exposure of normal human peripheral blood T cells to estradiol led to increased expression of the transcriptional repressor cyclic AMP response element modulator (CREM) alpha and suppression of IL-2 cytokine production (Moulton and Tsokos 2012).

Dendritic cells (DC) are initiators of the innate as well as adaptive immune responses and abundantly express the pattern recognition toll-like receptors (TLR). TLR7- and TLR9-deficient lupus-prone mice exhibit reduced disease, indicating that TLRs are important in lupus pathogenesis. DCs are defective in SLE in both humans and mice, exhibiting an overstimulated phenotype and function, with increased expression of major histocompatibility complexes (MHC) as well as costimulatory molecules CD80/86 (Kis-Toth and Tsokos 2010). Estrogen can modulate DC differentiation and function in several ways – alter the expression of MHC proteins, costimulatory molecules, or TLR; regulate cytokine production by DCs directly or indirectly via other cell types; and modulate migratory function through changes in cytokine or chemokine production. Furthermore, estrogen is required for the activation and differentiation of DCs, specifically those bearing features of a Langerhans cell-like DC (Nalbandian and Kovats 2005).

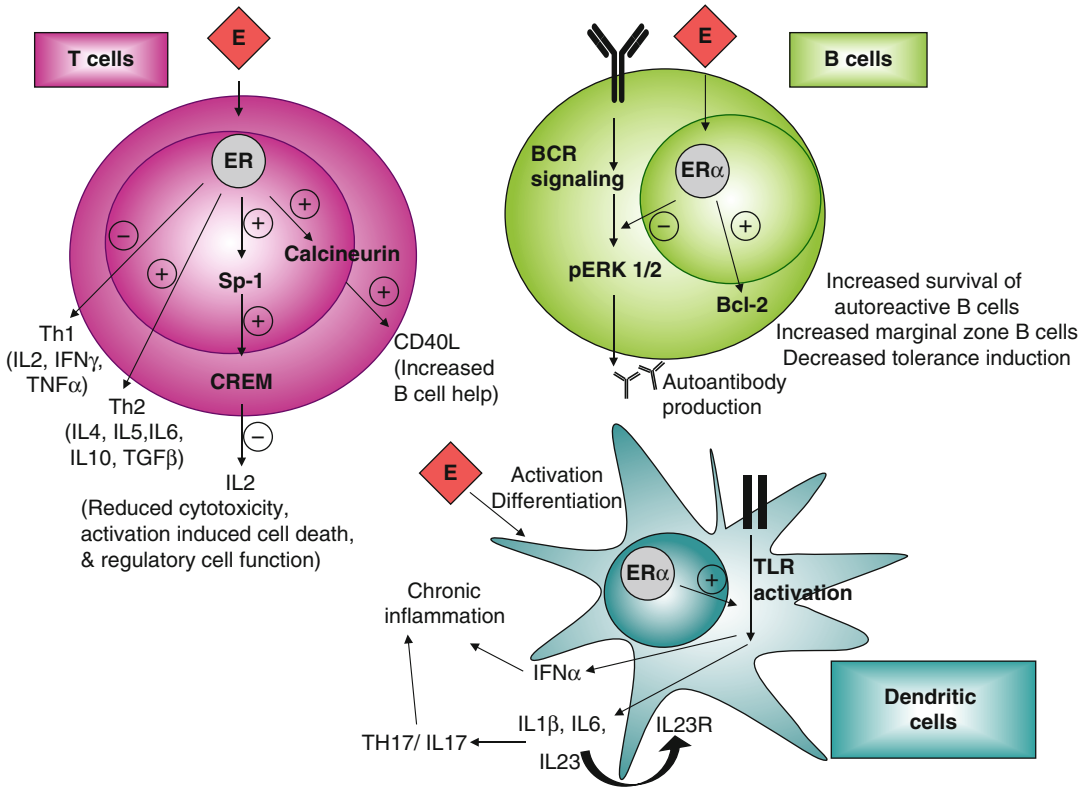
Besides the direct role of estrogen on the immune system, another interesting theory proposes that the regulatory mechanisms that normally control the estrogen-induced excitation of the immune response may be abnormal in SLE patients. To this end, DNA microarray analysis of genes expressed in the peripheral blood

mononuclear cells (PBMC) during the menstrual cycle of healthy women was compared to those from women with SLE and showed interesting differences. Specifically tumor necrosis factor receptor superfamily member 14 (TNFRSF14) (synonym: Herpes virus entry mediator HVEM) was found to be increased in correlation with increasing serum estrogen levels in healthy women but not in SLE patients. TNFRSF14 is a ligand for B and T lymphocyte attenuator (BTLA), an inhibitory receptor which dampens lymphocyte activation and is important in maintaining immune homeostasis. These results suggest that the mechanisms that regulate the immune-activating effects of estrogen may be defective in SLE patients (Sekigawa et al. 2010).

Estrogen Receptors

SLE patients experience increased disease activity and flares during pregnancy; however, in some studies, hormone levels were found to be lower in pregnant women with SLE as compared to healthy pregnant women. Thus, it is unclear if hormones alone determine disease activity (Walker 2011), rather the sensitivity of immune cells to hormones may be equally or more important. In this regard, the role of estrogen receptors (ER) and their contribution to disease are emerging. ER alpha (α) and beta (β) belong to the steroid hormone receptor superfamily and are intracellular receptors expressed in most immune cells including T cells, B cells, monocytes, and dendritic cells (Fig. 1). Estradiol diffuses through the cell membrane and binds to the ER which leads to the homo- or heterodimerization of the ER which then bind with high affinity to consensus estrogen response elements (ERE) sites within target genes, thus functioning as transcription factors to regulate gene expression. Besides the direct binding to target genes, ER-mediated regulation of gene transcription can occur indirectly via other proteins and can be ligand dependent or independent. The ER can act as a transcriptional co-activator and bind to other transcription factors such as specific protein 1 (Sp1), activator protein (AP)-1, or nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB). Besides the conventional intracellular ER, membrane-bound G protein-coupled receptors (GPR30) and cytoplasmic receptors have been identified which upon estrogen ligation can induce rapid intracellular calcium fluxing and intracellular signaling in various cell types. Interestingly, the ER can function in gene regulation in a ligand-independent manner – for example, extracellular stimuli such as insulin, IGF1, EGF, and TGF-β can lead to ER phosphorylation by MAP kinases and result in gene transactivation (Nilsson et al. 2001).

ER-α is required for hematopoietic and stromal development of a full-sized thymus in both male and female mice, and estrogen regulates CD4+CD8+ double-positive T-cell development which requires both ERα and ERβ. Estrogen can stimulate the production of regulatory T cells as it induced the expression of FoxP3 and IL-10 genes and promoted conversion of CD4 CD25- T cells to CD4CD25+ T regulatory cells, and this conversion was blocked by the ER antagonist ICI 182, 780. Several studies have highlighted the role of the ERs in lupus (Cunningham and Gilkeson 2011). In murine lupus studies, the ERα appears to be critical in disease pathogenesis; deficiency of ERα alone led to autoantibody production and reduced renal disease and prolonged survival in lupus-prone mice. Studies using estrogen receptor α- and β − deficient animals have shown that while both receptors regulate B-cell maturation, ERα engagement is responsible for the estrogen-induced dampening of the BCR signaling and B-cell selection and thus an important trigger for autoimmunity (Cohen-Solal et al. 2008; Cutolo et al. 2010). A ligand-independent role of the ER in the innate immune response in SLE has recently been proposed. Reduced TLR9-mediated inflammatory cytokine (IL-6, MCP-1, IL-1b, and IL-23) production was observed from DCs of both wild-type and lupus-prone ERα-deficient mice. Further, ERα-deficient DCs failed to upregulate IL-23R compared with wild-type DCs, suggesting that ERα may modulate the TLR induction of the IL-23/IL-17 pathway. These studies suggest



Systemic Lupus Erythematosus, Gender and Hormone Influences, Fig. 1 Diagram illustrating select roles of estrogen and estrogen receptors in the T cells, B cells, and dendritic cells in SLE (Adapted from Moulton and Tsokos 2012 with permission)

a cross talk between the ER and the TLR signaling that may occur in the absence of estrogen (Moulton and Tsokos 2012).

Hormone Therapy

While estrogen and estrogen receptors are potential culprits in SLE, estrogen has significant health benefits especially in bone metabolism and is crucial in osteoporosis treatment in postmenopausal women. Clinical trials have administered hormone replacement therapy in postmenopausal women and shown benefits in osteoporosis. The Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) studies, large multicenter randomized placebo-controlled clinical trials comprised of two separate arms – one of hormone replacement therapy and the other of combined oral contraceptive therapy in women with SLE. Results of these trials showed that hormone replacement therapy in postmenopausal women with SLE was associated with a small risk of mild to moderate flares. Historically, women with SLE were prohibited from using oral contraceptives for fear of exacerbating disease. However, the SELENA trials found that the use of combined estrogen-progesterone oral contraceptives in premenopausal women did not significantly increase the risk of flares in women with stable disease. However, caution must be exercised when considering hormone therapy in patients with SLE. For example, exogenous estrogen therapy should be avoided in patients with antiphospholipid antibodies due to the increased risk of thrombosis. Clinical trials have shown some utility of prescribing DHEA to patients in

terms of tapered prednisone doses, improved disease activity, and fewer flares. These studies indicate that the use of oral contraceptives and hormone therapy may be beneficial and warranted at least in some patients with SLE (Schwarz and Lohr 2006; Lateef and Petri 2012).

While the role of hormones and their receptors in autoimmunity and lupus disease pathogenesis is beginning to be uncovered, further molecular mechanistic studies are needed to understand and modulate these important contributors of disease.

Cross-References

- ▶ Novel Targets in Systemic Lupus Erythematosus
- ▶ Pregnancy in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Clinical Trials
- ▶ Systemic Lupus Erythematosus, Genetics
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Systemic Lupus Erythematosus, Treatment

References

Cohen-Solal JF, Jeganathan V, Hill L, Kawabata D, Rodriguez-Pinto D, Grimaldi C, Diamond B. Hormonal regulation of B-cell function and systemic lupus erythematosus. Lupus. 2008;17:528–32.

Cunningham M, Gilkeson G. Estrogen receptors in immunity and autoimmunity. Clin Rev Allergy Immunol. 2011;40:66–73.

Cutolo M, Brizzolara R, Atzeni F, Capellino S, Straub RH, Puttini PC. The immunomodulatory effects of estrogens: clinical relevance in immune-mediated rheumatic diseases. Ann N Y Acad Sci. 2010;1193:36–42.

Kassi E, Moutsatsou P. Estrogen receptor signaling and its relationship to cytokines in systemic lupus erythematosus. J Biomed Biotechnol. 2010;2010:317452.

Kis-Toth K, Tsokos GC. Dendritic cell function in lupus: independent contributors or victims of aberrant immune regulation. Autoimmunity. 2010;43:121–30.

Lahita RG. The role of sex hormones in systemic lupus erythematosus. Curr Opin Rheumatol. 1999;11:352–6.

Lahita RG. Gender and age in lupus. In: Lahita RG, editor. Systemic lupus erythematosus. 5th ed. San Diego: Elsevier; 2011.

Lang TJ. Estrogen as an immunomodulator. Clin Immunol. 2004;113:224–30.

Lateef A, Petri M. Hormone replacement and contraceptive therapy in autoimmune diseases. J Autoimmun. 2012;38:J170–6.

Moulton VR, Tsokos GC. Why do women get lupus? Clin Immunol. 2012;144:53–6.

Nalbandian G, Kovats S. Understanding sex biases in immunity: effects of estrogen on the differentiation and function of antigen-presenting cells. Immunol Res. 2005;31:91–106.

Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA. Mechanisms of estrogen action. Physiol Rev. 2001;81:1535–65.

Rider V, Abdou NI. Gender differences in autoimmunity: molecular basis for estrogen effects in systemic lupus erythematosus. Int Immunopharmacol. 2001;1:1009–24.

Salem ML. Estrogen, a double-edged sword: modulation of TH1- and TH2-mediated inflammations by differential regulation of TH1/TH2 cytokine production. Curr Drug Targets Inflamm Allergy. 2004;3:97–104.

Schwarz EB, Lohr PA. Oral contraceptives in women with systemic lupus erythematosus. N Engl J Med. 2006;354:1203–4 author reply 1203–1204.

Sekigawa I, Fujishiro M, Yamaguchi A, Kawasaki M, Inui A, Nozawa K, Takasaki Y, Takamori K, Ogawa H. A new hypothesis of the possible mechanisms of gender differences in systemic lupus erythematosus. Clin Exp Rheumatol. 2010;28:419–23.

Selmi C, Brunetta E, Raimondo MG, Meroni PL. The X chromosome and the sex ratio of autoimmunity. Autoimmun Rev. 2012;11:A531–7.

Walker SE. Estrogen and autoimmune disease. Clin Rev Allergy Immunol. 2011;40:60–5.

Systemic Lupus Erythematosus, Genetics

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Synonyms

Disseminated lupus erythematosus; Lupus; Lupus erythematosus; Systemic lupus erythematosus (SLE)

Definition

Systemic lupus erythematosus (SLE) is a complex, autoimmune disease characterized by the presence of antibodies to nuclear components. Genetic and environmental factors interact and contribute to the immunologic manifestations and development of disease, which can involve the skin, joints, hematologic system, kidneys, nervous system, mucosal and serosal membranes, and/or other organs.

Introduction

The importance of a genetic component in the pathogenesis of SLE has been implied for several decades based on early epidemiologic and genetic studies, particularly in multiplex families (Deng and Tsao 2010). Initial work confirmed several genetic signals utilizing methods such as linkage analysis and candidate gene association analysis. Our understanding of the genetic contribution to pathogenesis of disease in SLE has been greatly advanced, especially by recent genome-wide association (GWA) studies in populations of European and Asian ancestries (Sestak et al. 2011). In the vast majority of cases, SLE fits the common disease-common variant hypothesis, in which non-rare risk variants lead to a modest magnitude of risk (odds ratios of 1.1–2.5) and account for a portion of overall genetic susceptibility to disease. High-throughput genotyping platforms designed to identify common variants have been used in, to date, seven GWA studies (four in European-derived populations, three in Asians) and in a series of large-scale replication studies of individuals of European, Asian, African-American, and Amerindian/Hispanic descent. Many loci identified or confirmed by these studies are located either within or near genes encoding products with functional relevance to the pathogenesis of SLE (Guerra et al. 2012), providing an opportunity to potentially elucidate disease pathophysiology, predict disease course, and suggest drug targets. The nature of the complex pathophysiology of SLE has been underscored by many promising genetic susceptibility candidates from nearly all aspects of the immune system. In the summary below, those SLE susceptibility genes which have reached genome-wide significance, after correcting for multiple testing (Pe'er et al. 2008), are described.

Common SLE Susceptibility Genes Among Ancestral Backgrounds

Most identified SLE-associated risk loci are common genetic variations shared among multiple ancestral populations, suggesting their likely role in disease susceptibility and pathogenesis worldwide (Lee and Bae 2010; Scofield and Kaufman 2012). The variability between ancestral groups in frequencies of certain disease manifestations, including decreased risk of lupus nephritis in European backgrounds compared with Amerindian, Asian, and African-American descent (Richman et al. 2012), may be explained by divergent genetic effects of SLE susceptibility genes. For example, an increased effect size for several SLE loci has been observed in Amerindian compared with European ancestry (Sanchez et al. 2010) and in southern Europeans compared with central Europeans (Alonso-Perez et al. 2012). Variability in allelic frequency, differences in allelic linkage, the impact of gene-gene interactions, and particular environmental exposures may all contribute to such observed differences in ancestral groups, as well as limits of study design. Ongoing research in this area may ultimately provide important insight into both the common pathways leading to SLE manifestations, as well as more personalized risk profiles based on specific ancestral and genetic background.

Genotype-Phenotype Effects in SLE

Genotype effects may have an impact on SLE manifestations and disease course. For instance, an association between certain SLE susceptibility

genes, such as *ITGAM* (integrin alpha M or CD11b), *TNFSF4* (tumor necrosis factor superfamily, member 4), and *STAT4* (signal transducer and activator of transcription 4) (Sanchez et al. 2011), with the development of lupus nephritis has been suggested by several studies. Robust evidence has shown the *ITGAM* R77H risk allele conferring higher risk for lupus nephritis in case-only analyses of SLE patients of European and Asian ancestry. This integrin variant results in impaired phagocytosis and antigen-presenting cell adhesiveness to ICAMs (intercellular adhesion molecules) and complement factor iC3b, possibly resulting in decreased immunosuppressive effects and increasing SLE susceptibility. The possibility of "autoantibody propensity loci" which may further influence disease subphenotype is supported by the association between *IRF5* (interferon regulatory factor 5) risk haplotype, autoantibody production, and increased interferon (IFN)-α activity, whereby the impact of *IRF5* haplotypes on IFN activity is influenced by the presence of specific autoantibody subsets (Bronson et al. 2012). There may, in fact, be baseline increases in IFN activity based on *IRF5* haplotype leading to increased risk for development of autoantibodies and autoimmune disease (Rullo et al. 2010). Learning more loci to account for heritability will enhance the link between genotype and phenotype in SLE.

Immunological Pathways Affected by SLE Susceptibility Variants

Current understanding of SLE pathogenesis can group several identified SLE susceptibility genes into major immunological pathways. Certain immunological pathways, including the well-established *HLA* genetic region (Relle and Schwarting 2012), are common to multiple autoimmune disorders. A growing number of additional SLE susceptibility genes also predispose to other related conditions. An example to highlight is *TNFAIP3* (tumor necrosis factor, alpha-induced protein 3), which encodes the ubiquitin-modifying enzyme A20, a control mechanism that can terminate prolonged activation of inflammatory responses. The widely expressed A20, a cytoplasmic enzyme, regulates NF-κB (nuclear factor-KappaB)-driven gene expression after TNFR (tumor necrosis factor receptor), IL-1R (interleukin 1 receptor), and TLR (toll-like receptor) signaling, leading to altered dendritic cell homeostasis. Several SNPs in the A20 genomic locus have been found to be associated with chronic inflammation and increased susceptibility to SLE, rheumatoid arthritis, systemic sclerosis, type 1 diabetes, Crohn's disease, and multiple sclerosis (Vereecke et al. 2011).

DNA Degradation, Apoptosis, and Clearance of Cellular Debris

An abundance of self-antigens due to inappropriate disposal of intracellular constituents or infectious agents can initiate and drive aberrant immune responses in SLE. Several variants of genes related to these pathways contribute to both monogenic and polygenic forms of SLE (Table 1). Some examples include:

- *TREX1* (three prime repair exonuclease 1): Deep sequencing of this gene identified novel mutations in patients with SLE but not controls; common variations of *TREX1* are also associated with SLE (Bronson et al. 2012). Deficiency of TREX1, an exonuclease involved in cell death, DNA degradation, and cellular response to oxidative damage, typically leads to Aicardi-Goutieres syndrome, a neurologic condition characterized by increased IFN-α and varied autoimmune phenomenon.
- *ACP5* (acid phosphatase 5, tartrate resistant): Mutations in this gene lead to elevated IFN-α activity and a spectrum of autoimmune diseases including SLE (Bronson et al. 2012). Deficiency of the gene product TRAP (tartrate-resistant acid phosphatase), a protein that functions in lysosomal digestion, is known to cause the syndrome of spondyloenchondrodysplasia.

Systemic Lupus Erythematosus, Genetics, Table 1 Pathway-associated genes: DNA degradation, apoptosis, and clearance of cellular debris

Candidate gene	Putative functional significance	Associated SLE manifestations
ACP5	Overactivation of IFN-α	ANA and anti-dsDNA antibodies, renal disorder, arthritis, thrombocytopenia
TREX1	Removal of nucleic acids	Neuropsychiatric SLE
DNASE1/1L3	Clearance of apoptotic debris	N.D.
ATG5	Formation of autophagosomes	N.D.

N.D. not described, *DNASE1/1L3* deoxyribonuclease 1, *ATG5* autophagy related 5

Immune Complex Clearance and Phagocytosis

Genetic variants that result in lower levels of complement components, a classic feature of SLE pathophysiology, or abnormalities of apoptotic cell clearance may lead to the initiation of the autoimmune response (Table 2). Some examples to highlight include:

- *C1Q*; *C4A/B*: The incidence of SLE or lupus-like manifestation in individuals with a complete deficiency, due to a homozygous mutation, in one of the classical complement pathway genes ranges from 10 % to 93 %, with an extremely strong genetic risk for SLE and glomerulonephritis in young people who are C1q-deficient (93 % penetrant) and deficiency in both C4A and C4B genes (75 % penetrant) individuals (Chen et al. 2010).
- FcγR (Fc fragment of IgG, low affinity receptor) gene variants: With function in immune complex clearance, the role of FcγR variants to risk of SLE is highlighted by several variants, including H131R of *FCGR2A*, F158V of *FCGR3A*, and I232T of *FCGR2B*, which have been associated with SLE susceptibility in several ancestral populations and with specific disease profiles (Li et al. 2009). Investigation of the gene structure of the Fc receptor gene family region is complicated by the presence of gene duplications and copy-number variations. Inconsistencies between FcγRs genetic studies in SLE have been attributed to ethnic differences and disease heterogeneity, as well as genotyping error. Further investigation of functional consequences of the FcγR gene variants in SLE is warranted and will help to characterize their contributions to disease pathogenesis.

Systemic Lupus Erythematosus, Genetics, Table 2 Pathway-associated genes: immune complex processing and phagocytosis

Candidate gene	Putative functional significance	Associated SLE manifestations
C1Q	Reduced handling of apoptotic cell debris and immune complexes	Renal disorder, photosensitivity, neurological disorder
C1R/C1X	Early key regulators of complement cascade	Renal disorder
C2	Early key regulator of complement cascade	Renal disorder
C4A/B	Clearance of immune complex; propagating complement cascade	Arthritis, renal disorder
FCGR2A	Reduced immune complex clearance	Renal disorder, antiphospholipid syndrome, malar rash
FCGR3A/B	Reduced immune complex clearance	Renal disorder
ITGAM	Leukocyte activation and migration	Discoid rash; arthritis; renal, neurological, hematological, and immunological disorders

TLR, IFN, and NF-κB Pathways

Immune complexes consisting of nucleic acid self-antigens are known to bind the pattern recognition receptors TLR7 and TLR9. These receptors activate transcription factors, leading to activation of the type I interferon pathway, an important early proinflammatory mechanism utilized as a response to viral infection.

Systemic Lupus Erythematosus, Genetics, Table 3 Pathway-associated genes: TLR and type I IFN signaling

Candidate gene	Putative functional significance	Associated SLE manifestations
TLR7	Bind nucleic acid-containing immune complexes, induce interferon-alpha response	Anti-RBP antibodies
IRF5	Transcription factor that induces interferon-alpha and downstream gene production	Anti-dsDNA and anti-Ro antibodies
IRF7	Transcription factor that induces interferon-alpha and downstream gene production	Anti-dsDNA and anti-Sm antibodies, immunological disorder
IRF8	B cell differentiation	N.D.
IRAK1	Suppression of TLR response	N.D.
TYK2	Interaction with IFN receptor	N.D.
PRDM1	Hyperproliferation of T cells	N.D.
STAT4	Immune cell signal transduction	Early age at disease onset, renal disorder, anti-dsDNA, antiphospholipid syndrome
TREX1	Removal of nucleic acids	Neuropsychiatric SLE
ACP5	Overactivation of IFN-α	ANA and anti-dsDNA antibodies, renal disorder, arthritis, thrombocytopenia

IRAK1 interleukin-1 receptor-associated kinase 1, *PRDM1* PR domain zinc finger protein 1

Systemic Lupus Erythematosus, Genetics, Table 4 Pathway-associated genes: NF-κB signaling

Candidate gene	Putative functional significance	Associated SLE manifestations
IRAK1	Suppression of TLR response	N.D.
TNFAIP3	Restricts TNF and NF-κB signals	Renal and hematological disorders
TNIP1	NF-κB inhibition	Photosensitivity, vasculitis
UBE2L3	Regulation of the TLR response	Anti-dsDNA antibodies
SLC15A4	NF-κB signaling pathway regulation	Discoid rash
PRKCB	B cell activation, apoptosis induction	N.D.

RBP ribosomal binding protein (included: Sm/RNP, SSA/SSB)

The overly abundant activation of this and downstream pathways in patients with SLE, triggered by either viral or endogenous nucleic acids, leads to the persistent production of interferon-inducible, antiviral genes (Niewold 2011). Several variants have been associated with risk of SLE (Table 3); for example, a functional 3′ untranslated region (UTR) SNP of the X-linked *TLR7* that confers elevated TLR7 expression and an increased IFN response has been associated with SLE in East Asians (Shen et al. 2010), which was subsequently confirmed in European-American, African-American, and Hispanic populations (Deng et al. 2011). Variation in genes coding for transcription factors downstream of TLRs, including IRF5, IRF7, and IRF8, has been associated with SLE susceptibility (Bronson et al. 2012). Several additional genes within or downstream of the type I IFN pathway have been associated with risk of SLE (Table 3), including *STAT4*, *IFIH1* (interferon induced with helicase C domain 1), *TYK2* (tyrosine kinase 2), and *PRDM1* (PR domain zinc finger protein 1) (Bronson et al. 2012). Genes that play a role in the NF-κB pathway downstream of TLR engagement have also been associated with increased SLE susceptibility in multiple ancestries and are included in the list below (Table 4).

Immune Cell Signaling and Function

After recognition of self-DNA or RNA from dying or damaged cells, autoreactive B cells produce autoantibodies that form immune complexes and drive other proinflammatory

Systemic Lupus Erythematosus, Genetics, Table 5 Pathway-associated genes: lymphocyte function and signaling

Candidate gene	Putative functional significance	Associated SLE manifestations
FCGR2B	232T risk allele reduces inhibitory activity of BCR signaling	232T and risk of SLE susceptibility has been established only in patients of Asian descent
BLK	B cell development	Antiphospholipid syndrome, anti-dsDNA antibodies
LYN	B cell activation	Discoid rash, hematological disorder
BANK1	Attenuates B cell proliferation and survival	N.D.
CSK	Modify kinase activation	N.D.
PRDM1	Hyperproliferation of T cells	N.D.
ETS1	B and T cell differentiation	Early age at disease onset
IKZF1	Perturbed TH1/TH2 balance	Malar rash, renal disorder
AFF1	Development of lymphocytes	N.D.
RASGRP3	Lymphocyte activation	Malar rash, discoid rash, ANA
IL10	Promotes B cell hyperactivity and autoantibody production, downregulates antigen presentation	aCL-IgM, anti-Sm, anti-SSA antibodies; discoid rash; renal and neurological disorders
IL21	TH17 cell response	Hematological disorder
NCF2	Increases B cell differentiation	N.D.
PRKCB	B cell activation, apoptosis induction	N.D.
HLA-DR2 & DR3	Peptide presentation to CD4+ T cells	Anti-Ro/La and anti-dsDNA antibodies
MSH5	Meiotic recombination	N.D.
IRF8	B cell differentiation	N.D.
PTPN22	Reduced removal of autoreactive B cells	N.D.
TNFSF4	Co-stimulation for inhibition of T regulatory cells	N.D.
CD44	Lymphocyte activity	Thrombocytopenia
TYK2	Interaction with IFN receptor	N.D.
STAT4	Immune cell signal transduction	Early age at disease onset, renal disorder, anti-dsDNA, antiphospholipid syndrome

BLK B lymphoid tyrosine kinase, *LYN* tyrosine protein kinase LYN, *BANK1* B cell scaffold protein with ankyrin repeats 1, *CSK* tyrosine protein kinase CSK, *ETS1* Ets-1 protein or p54, *IKZF1* IKAROS family zinc finger 1, *AFF1* AF4/FMR2 family member 1, *RASGRP3* RAS guanyl-releasing protein 3, *IL10* interleukin 10, *IL21* interleukin 21, *PRKCB* protein kinase C, beta, *MSH5* MutS protein homolog 5, *PTPN22* protein tyrosine phosphatase, non-receptor type 22

responses. T cells and additional antigens can further stimulate B cells, leading to hyperactivity with increased autoantibody production. Gene variants involved in B and T cell signaling have been associated with SLE susceptibility in multiple ancestral backgrounds (Vaughn et al. 2012), as described in the list below (Table 5). Additionally, the production of superoxide by NADPH oxidase in leukocytes stimulated by autoantigens may be affected by an amino acid change (H389Q) in *NCF2* (neutrophil cytosolic factor 2), an SLE susceptibility gene encoding a subunit of the NADPH oxidase enzyme, implicating a role for decreased reactive oxygen species in SLE pathogenesis (Jacob et al. 2012).

Neutrophils and monocytes may play an important role in the pathogenesis of SLE, particularly in the formation of NETs (neutrophil extracellular traps) containing DNA and neutrophil-derived proteins which trigger IFN-α and can directly damage cells (Dorner 2012). Variants in genes with function in adhesion and migration of both cell types have been associated with SLE susceptibility in multiple ancestries (Table 6), specifically R77H *ITGAM* and the *ICAM* loci, encoding an ITGAM ligand (Kim et al. 2012).

Systemic Lupus Erythematosus, Genetics, Table 6 Pathway-associated genes: neutrophil and monocyte function and signaling

Candidate gene	Putative functional significance	Associated SLE manifestations
ITGAM	Leukocyte activation and migration	Discoid rash; arthritis; renal, neurological, hematological, and immunological disorders
ICAMs	Ligands for cellular adhesion	N.D.
FCGR2B	B cell differentiation	N.D.
FCGR3A/B	Reduced immune complex clearance	Renal disorder, antiphospholipid syndrome, malar rash
IL10	Downregulates immune responses	aCL-IgM, anti-Sm, anti-SSA antibodies; discoid rash; renal and neurological disorders
IRF8	Lymphocyte differentiation	N.D.

Conclusion

The identification of susceptibility genes for the development of SLE can help to understand both disease pathogenesis and tolerance breakdown in human immunology. To date, several important immunologic pathways have been implicated as responsible for susceptibility to disease, including DNA degradation, apoptosis, immune complex clearance, immune cell signaling and function, and inappropriate activation of the IFN, TLR, and NF-κB pathways. Research probing the dysfunction within these pathways has led to novel medications that may have therapeutic impact in SLE, including anti-IFN-α and anti-BLyS therapies. Several functional variants within these pathways have been determined and explored, such as risk alleles of *TLR7* and *IRF5* which exert control over IFN activity, enhancing our understanding of possible links between genotype and specific disease manifestations. Investigating all genetic backgrounds, including Amerindian, Hispanic, and African-American will lead to discovery of additional risk loci and ultimately greater understanding of common pathways to development of SLE. The development of drug therapy in SLE will be driven by the ongoing discovery of major susceptibility genes which are common among ancestral backgrounds. Next-generation sequencing technology such as whole genome sequencing and whole exome sequencing may help discover novel rare and functional risk variants, as well as provide further insight into SLE pathogenesis and molecular mechanisms of immune tolerance.

Cross-References

- ▶ Complement in Rheumatic Diseases
- ▶ Discoid SLE
- ▶ Epigenetics in Autoimmunity
- ▶ Juvenile Diseases: SLE in Children
- ▶ Lupus Nephritis and Novel Therapies, Pathogenesis
- ▶ NF-κB
- ▶ Novel Targets in Systemic Lupus Erythematosus
- ▶ PTPN22
- ▶ Rheumatoid Arthritis, Genetics
- ▶ Skin in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Animal Models
- ▶ Systemic Lupus Erythematosus, Autoantibodies
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Systemic Lupus Erythematosus, Clinical Trials
- ▶ Systemic Lupus Erythematosus, Gender and Hormone Influences
- ▶ Systemic Lupus Erythematosus, Pathogenesis
- ▶ Systemic Lupus Erythematosus, Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Alonso-Perez E, Suarez-Gestal M, Calaza M, Sebastiani GD, Pullmann R, Papasteriades C, et al. Bias in effect size of systemic lupus erythematosus susceptibility loci across Europe: a case–control study. Arthritis Res Ther. 2012;14(2):R94.

Bronson PG, Chaivorapol C, Ortmann W, Behrens TW, Graham RR. The genetics of type I interferon in systemic lupus erythematosus. Curr Opin Immunol. 2012;24(5):530–7.
Chen M, Daha MR, Kallenberg CG. The complement system in systemic autoimmune disease. J Autoimmun. 2010;34(3):J276–86.
Deng Y, Tsao BP. Genetic susceptibility to systemic lupus erythematosus in the genomic era. Nat Rev Rheumatol. 2010;6(12):683–92.
Deng Y, Zhao J, Tan WF. Association of a functional variant in TLR7 with systemic lupus erythematosus and rheumatoid arthritis in multiple ancestries. Arthritis Rheum. 2011;63(966):s966.
Dorner T. SLE in 2011: deciphering the role of NETs and networks in SLE. Nat Rev Rheumatol. 2012;8(2):68–70.
Guerra SG, Vyse TJ, Cunninghame Graham DS. The genetics of lupus: a functional perspective. Arthritis Res Ther. 2012;14(3):211.
Jacob CO, Eisenstein M, Dinauer MC, Ming W, Liu Q, John S, et al. Lupus-associated causal mutation in neutrophil cytosolic factor 2 (NCF2) brings unique insights to the structure and function of NADPH oxidase. Proc Natl Acad Sci USA. 2012;109(2):E59–67.
Kim K, Brown EE, Choi CB, Alarcon-Riquelme ME, Kelly JA, Glenn SB, et al. Variation in the ICAM1-ICAM4-ICAM5 locus is associated with systemic lupus erythematosus susceptibility in multiple ancestries. Ann Rheum Dis. 2012;71:1809–14.
Lee HS, Bae SC. What can we learn from genetic studies of systemic lupus erythematosus? Implications of genetic heterogeneity among populations in SLE. Lupus. 2010;19(12):1452–9.
Li X, Ptacek TS, Brown EE, Edberg JC. Fcgamma receptors: structure, function and role as genetic risk factors in SLE. Genes Immun. 2009;10(5):380–9.
Niewold TB. Interferon alpha as a primary pathogenic factor in human lupus. J Interferon Cytokine Res. 2011;31(12):887–92.
Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genet Epidemiol. 2008;32(4):381–5.
Relle M, Schwarting A. Role of MHC-linked susceptibility genes in the pathogenesis of human and murine lupus. Clin Dev Immunol. 2012;2012:584374.
Richman IB, Taylor KE, Chung SA, Trupin L, Petri M, Yelin E, et al. European genetic ancestry is associated with a decreased risk of lupus nephritis. Arthritis Rheum. 2012;64(10):3374–82.
Rullo OJ, Woo JM, Wu H, Hoftman AD, Maranian P, Brahn BA, et al. Association of IRF5 polymorphisms with activation of the interferon alpha pathway. Ann Rheum Dis. 2010;69(3):611–7.
Sanchez E, Webb RD, Rasmussen A, Kelly JA, Riba L, Kaufman KM, et al. Genetically determined Amerindian ancestry correlates with increased frequency of risk alleles for systemic lupus erythematosus. Arthritis Rheum. 2010;62(12):3722–9.
Sanchez E, Nadig A, Richardson BC, Freedman BI, Kaufman KM, Kelly JA, et al. Phenotypic associations of genetic susceptibility loci in systemic lupus erythematosus. Ann Rheum Dis. 2011;70(10):1752–7.
Scofield RH, Kaufman KM. Mapping susceptibility gene in systemic lupus erythematosus. Methods Mol Biol. 2012;900:11–24.
Sestak AL, Furnrohr BG, Harley JB, Merrill JT, Namjou B. The genetics of systemic lupus erythematosus and implications for targeted therapy. Ann Rheum Dis. 2011;70(Suppl 1):i37–43.
Shen N, Fu Q, Deng Y, Qian X, Zhao J, Kaufman KM, et al. Sex-specific association of X-linked toll-like receptor 7 (TLR7) with male systemic lupus erythematosus. Proc Natl Acad Sci USA. 2010;107(36):15838–43.
Vaughn SE, Kottyan LC, Munroe ME, Harley JB. Genetic susceptibility to lupus: the biological basis of genetic risk found in B cell signaling pathways. J Leukoc Biol. 2012;92(3):577–91.
Vereecke L, Beyaert R, van Loo G. Genetic relationships between A20/TNFAIP3, chronic inflammation and autoimmune disease. Biochem Soc Trans. 2011;39(4):1086–91.

Systemic Lupus Erythematosus, Pathogenesis

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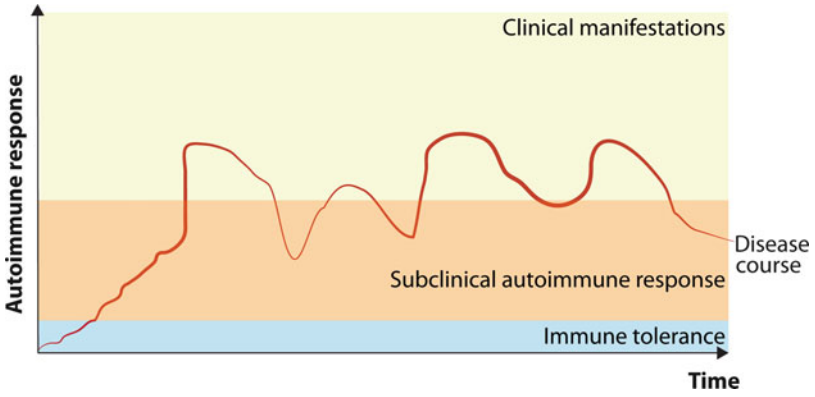
Synonyms

Cause of SLE

Definition

The mechanisms or pathological processes by which a collection of inherited and acquired abnormalities cause SLE.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects mostly women. Patients with SLE have laboratory abnormalities that indicate that immune tolerance towards self-antigens has broken and



Systemic Lupus Erythematosus, Pathogenesis, Fig. 1 *Development of lupus involves a subclinical chronic autoimmune response and acute bouts of organ inflammation*. Patients with SLE develop a chronic autoimmune response that can be detected by the presence of autoantibodies. This response precedes and underlies the obvious clinical manifestations that occur when products of the autoimmune response cause local inflammation

heterogeneous clinical manifestations that vary according to the involvement of different organs and systems.

One of the characteristics of SLE that has baffled clinicians and has complicated its understanding is that patients with lupus differ very much from each other. Moreover, different pathogenic mechanisms underlie each clinical manifestation. For example, lupus nephritis is caused by the deposition of immune complexes in the renal glomeruli and by the infiltration of lymphocytes into the renal interstitium, whereas thrombocytopenia is caused by antiplatelet antibodies.

In contrast to organ-specific autoimmune diseases (e.g., autoimmune thyroid disease) that are driven by immune responses against components of the affected organ, in lupus most manifestations occur when cellular or soluble components of the immune system activated during the course of a chronic autoimmune response directed towards widely expressed molecules gain access to tissues and cause local inflammation. Lupus nephritis is not caused by an immune response against components of the glomeruli; it is triggered by the deposition of immune complexes in the glomerular mesangium and basement membrane. These immune complexes are not formed by anti-renal antibodies but rather by various autoantibodies that deposit in the glomeruli. Ensuing activation of complement and neutrophils causes glomerulonephritis which is probably modulated by the response of the kidney to the presence of immune complexes and local inflammation (Bagavant and Fu 2009).

Patients with SLE develop a chronic autoimmune response directed mostly against ubiquitously expressed intracellular antigens (e.g., DNA and ribonucleoproteins). This response is present in most – if not all – patients with SLE and causes no clinical symptoms directly. It becomes evident as antinuclear antibodies (ANA). When by-products of this response gain access to tissues (e.g., DNA-anti-DNA immune complexes deposit in the glomeruli), they cause local inflammation that manifests clinically. Thus, SLE has a common, mostly subclinical component (a chronic autoimmune response against widely expressed intracellular antigens) that is necessary but not sufficient to cause the disease and a second heterogeneous component directly involved in causing clinical manifestations.

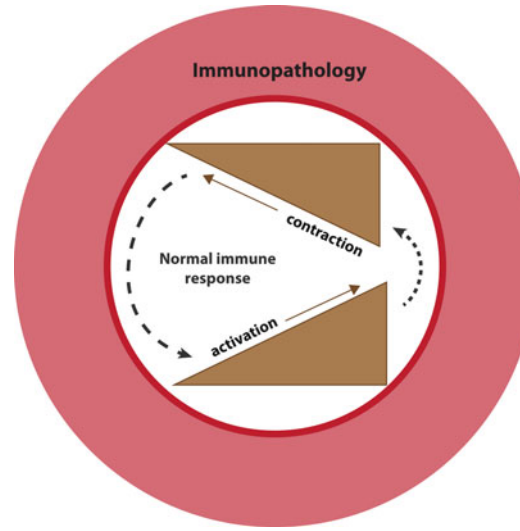
In this regard, the pathogenesis of SLE involves a first step when self-tolerance is broken and the immune system engages a chronic autoimmune response and a second step that determines when and where the products of the underlying autoimmune response will instigate inflammation (Fig. 1).

Genetic and Environmental Factors

Why some individuals develop SLE is still a matter of debate (Tsokos 2011). Early genetic studies demonstrated that SLE has a genetic component: it shows familial aggregation and high concordance rates among twins, and its prevalence and severity vary among different ethnic groups (see ▶ Systemic Lupus Erythematosus, Genetics). Accordingly, genome-wide association studies (GWAS) have identified several markers that are more commonly found in patients with SLE than in healthy controls. Not surprisingly, most of these markers are within (or in close proximity to) genes that encode for proteins involved in the immune response. These markers (single nucleotide polymorphisms or SNP) are mostly found in noncoding DNA regions, and the effects most of them exert on the immune system are still poorly understood. They probably modify the function of the gene they are associated with in a subtle manner. It is important to emphasize that although these SNPs are more common in patients with SLE, they are also frequent in healthy individuals. Therefore, the presence of each is predicted to have a small impact in immune function (since they are found in healthy individuals). Accordingly, the current hypothesis is that lupus is more likely to develop in persons who carry numerous susceptibility SNPs. Each SNP may contribute in a small way to an immune system more prone to inflammation or autoreactivity, but only the combination of several will exceed the threshold of susceptibility to lupus. This model also predicts that different combinations of susceptibility SNPs will be associated with different clinical phenotypes. In this sense, the combination of hereditary traits that fine-tune the immune behavior of an individual can also, under certain circumstances, make that individual prone to develop lupus. How these hereditary traits modulate the immune system and, importantly, how they interact with each other are subject of intense scrutiny.

A great majority of SLE patients are women in the childbearing age; lupus is less common before puberty and after menopause, and in those ages, the difference in frequency between men and women is less marked. Therefore, female sex and in particular female hormones are considered a strong predisposing factor for lupus. Again, a variety of studies have shown that hormones exert broad effects in the immune system, but the exact mechanisms through which female hormones promote autoimmunity are incompletely understood (see ▶ Systemic Lupus Erythematosus, Gender and Hormone Influences).

Thus, the current paradigm is that hereditary factors shape the immune system in such a way that it becomes susceptible of losing immune tolerance upon encountering unknown trigger (s). At some point during the life of the individual with lupus, an external agent is thought to activate the immune response, driving it over the tolerance threshold (Fig. 2). Agents able to cause cellular damage and activate the immune responses such as UV light and in particular viral



Systemic Lupus Erythematosus, Pathogenesis, Fig. 2 *A dysregulated immune response can produce immune-mediated pathology*. The immune system gets activated and expands upon antigenic stimulation. In healthy individuals, immunopathology is avoided by restricting the magnitude and the duration of the immune response and by ensuring that most of the immune cells that have been produced during the response are eliminated during the contraction phase. If the mechanisms that limit the intensity of the immune response are faulty, the elements of the immune response will cause organ damage

infections have been proposed as culprits. Importantly, disease development and tolerance loss probably represent a gradual process induced by multiple events (Liu and Davidson 2012). This is supported by the finding that antibodies against self-antigens are produced in patients with lupus several years before clinical disease is evident and that the variety and amount of these autoantibodies increases slowly until disease becomes overt. Therefore, loss of tolerance, manifested by the production of an immune response against self-antigens, precedes by years the development of organ and tissue injury. This suggests that the autoimmune response is initially of small magnitude but that – in persons who eventually develop disease – it slowly increases until its products (i.e., autoantibodies, cytokines, activated cells) become pathogenic by virtue of specificity (e.g., development of anti-red blood cell antibodies that cause autoimmune hemolytic anemia) or quantity (e.g., immune complexes that deposit in the renal glomeruli) (Fig. 1).

An important concept to understand this hypothesis is the continuous remodeling of the immune system. Lymphocytes (T cells and B cells) activated during an immune response proliferate and increase their affinity to the antigens that triggered the response. Therefore, the immune system repertoire adjusts after each immune bout. By the time lupus becomes clinically apparent, the immune system of an affected individual probably harbors a relatively large number of autoreactive T and B cells that have expanded and gradually accumulated cells that by virtue of number and functionality are able to produce a highly specific and powerful self-aimed immune response. A pathogenic immune response will thus slowly develop in a susceptible person driven by encounters with environmental triggers (Shlomchik et al. 2001).

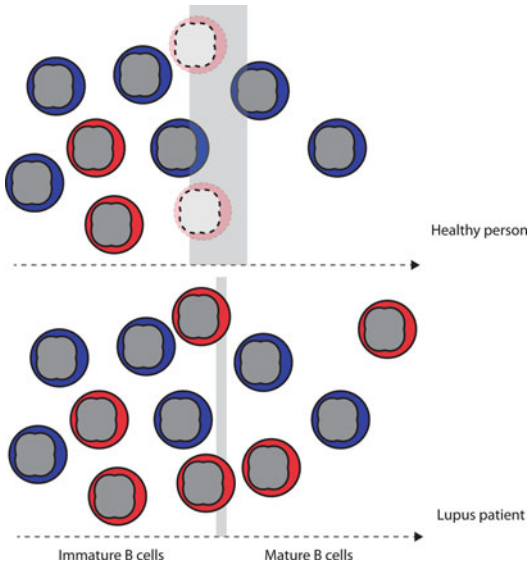
Innate Immune System

The innate immune system, and in particular antigen-presenting cells (APC), probably plays a major role in the development of SLE. APCs are responsible for detecting pathogens and for initiating and modulating the ensuing immune response. Moreover, they handle self-antigen-rich cellular debris that should be presented to the adaptive immune system in a noninflammatory fashion (i.e., as innocuous self-antigens). The responses of APCs to antigens are determined by a number of factors such as the local abundance of cytokines and signals that indicate the presence of a noxious agent (i.e., molecules associated to infectious agents or produced by stressed cells). These cues induce functional and phenotypic changes in the APC and deeply affect the manner it presents the antigen to T cells. Normally, self-antigens are presented as such, and T cells able to recognize them are deactivated; this process maintains self-tolerance. When antigens are associated to pathogens, antigen presentation elicits the activation and proliferation of T cells, triggering an efficient, proinflammatory immune response that will eliminate the source of the antigen. Evidence from animal models and from patients that lack key molecules responsible for the clearance of cellular debris indicates that when the load of self-antigens is not adequately handled by the innate immune system, autoantigens may elicit immune responses. In fact, apoptotic cells are thought to be an important source of autoantigens in SLE. This process may represent a primary defect that promotes the development of lupus but may also facilitate established disease. Patients with SLE have antibodies that are directed against self-constituents. Therefore, apoptotic cells may become coated by these autoantibodies and their recognition by the immune system affected. An APC will not interpret an antibody-coated particle as self. In this way, autoantibodies contribute to the amplification of the autoimmune pathology and to the breach in tolerance towards self-antigens. Another factor that may play a role in patients with ongoing disease is that the presence of an inflammatory environment may facilitate the activation of APCs and their presentation of self-antigens in a proinflammatory manner. For instance, immune complexes present in patients with SLE can stimulate APC activation through immunoglobulin receptors.

Humoral Immune Response

Each B cell creates a unique antibody through a process of random gene rearrangement. This process allows the production of a large array of different antibodies, some of them autoreactive. After immature B cells create and express a unique immunoglobulin (as B cell receptor), they undergo a selection process designed to eliminate self-reactive B cells before their maturation into immunocompetent cells. This process has been shown to be altered in patients with SLE. Thus, more autoreactive B cells are present in patients with SLE (Fig. 3).

Autoantibodies are the most commonly observed immune alteration in patients with lupus (see ▶ Systemic Lupus Erythematosus, Autoantibodies). SLE autoantibodies have high affinity and have been produced by cells that have participated in a full-blown immune response.



Systemic Lupus Erythematosus, Pathogenesis, Fig. 3 *Autoreactive B cells are inadequately eliminated in patients with SLE*. Since immunoglobulin production depends on random genetic rearrangement, a fraction of B cells creates autoreactive immunoglobulins. These potentially harmful cells (*red cells*) are eliminated before becoming mature (*upper panel*). This process is defective in patients with lupus (*lower panel*) that have more mature autoreactive cells in their B cell repertoire

Although the autoantibody response in SLE is broad and directed against numerous specificities, several hallmark antigens are intranuclear complexes comprised of nucleic acids and proteins. This might be important from the pathogenic point of view, since B cells can be stimulated by DNA and RNA through toll-like receptors. In fact, evidence obtained in animal models of lupus suggests that nucleic acids contained in immune complexes can deliver a second signal to the B cell, complementary to the signal conveyed by the immunoglobulin. Thus, nucleic acid-containing immune complexes might be able stimulate B cells without the need of T cell-derived help providing an alternative pathogenic amplification pathway independent of T cell help and regulation.

Autoantibodies produced by patients with SLE have been shown to be pathogenic through different mechanisms. Autoantibodies form immune complexes with antigens derived from injured or apoptotic cells and can be detected in sera of patients with lupus. They can deposit in certain vascular beds (e.g., renal glomeruli) where they induce inflammation by activating complement and engaging immunoglobulin receptors in neutrophils and other inflammatory cells. When present, anti-red blood cell and antiplatelet antibodies cause hemolytic anemia and autoimmune thrombocytopenia. Autoantibodies can also induce tissue damage by mechanisms different than immune complex deposition. In experimental models, autoantibodies isolated from patients with lupus have been shown to bind to placenta and ischemic intestine and trigger inflammatory responses that cause, respectively, fetal resorption and ischemia-reperfusion-mediated tissue injury. Autoantibodies can also bind to surface receptors and alter cellular function. For example, some anti-DNA antibodies cause neuronal apoptosis by promoting the prolonged activation of glutamate receptors, while anti-T lymphocyte antibodies can significantly alter T cell function and cytokine production by activating intracellular pathways that modify the activation of transcription factors. In pregnant patients with

lupus, autoantibodies (e.g., anti-Ro) may cross the placenta and cause fetal damage (Diamond and Volpe 2012).

Cellular Immune Response

T cells regulate immune responses through the release of cytokines and the expression of membrane-bound factors that affect the behavior of other immune and nonimmune cells. They also induce cellular apoptosis in cells affected by intracellular pathogens such as viruses. In lupus patients, the function of T cells is altered: their activation process and gene expression profile are abnormal. This is reflected in an aberrant cytokine production profile and altered effector functions (Crispin et al. 2010).

When activated, lupus-derived T cells exhibit an abnormally rapid and elevated surge in intracellular calcium accompanied by increased phosphorylation of signaling proteins. This is caused by alterations in the composition of proteins associated to the T cell receptor and to structural changes in the plasma membrane. These changes have profound consequences, because when a lupus T cell is stimulated by an antigen, its response, in terms of gene expression and cellular behavior, is abnormal. Gene transcription is controlled by a multilayered process that includes DNA methylation and posttranslational histone modifications. DNA methylation is decreased in T cells from lupus patients. This alteration is responsible for the overexpression of a number of genes. Interestingly, two drugs that cause drug-induced lupus (i.e., procainamide and hydralazine) induce DNA demethylation. Therefore, an altered activation process along with defects in gene regulation affects T cell function in patients with SLE.

One of the most obvious T cell defect is the skewed cytokine production. Since cytokines are probably the most important conduit used by T cells to deliver signals, aberrant cytokine production implies faulty immune regulation. Production of interleukin 2 (IL-2) is impaired in SLE T cells. This defect has been associated with several T cell abnormalities that not only determine autoimmune pathology but also impair the development of protective immune responses against pathogens. On the other hand, decreased IL-2 has been postulated to be involved in quantitative and qualitative abnormalities in regulatory T cells. Moreover, activation-induced cell death, a mechanism that allows the contraction of expanded clones of activated lymphocytes at the end of an immune response, also depends on the presence of IL-2. On the other hand, production of IL-17, a proinflammatory cytokine, is increased in patients with lupus. Importantly, IL-17-producing T cells infiltrate kidneys from patients with lupus nephritis causing local inflammation.

T cells regulate the production of antibodies by directly stimulating B cells and by producing cytokines that modulate B cell function. T cells from patients with SLE exhibit an enhanced capacity to stimulate B cells. Moreover, characteristics of the autoantibodies found in patients with SLE indicate that the B cells that produce them have received T cell stimulation (Craft 2011).

In summary, SLE is a complex disease that develops over time in individuals who harbor an inherited predisposition kindled by undefined environmental factors. Discrete defects in virtually every cell of the immune system contribute to shape an immune system that is more inflammatory and more prone to tolerance breaches. At some point, the immune system crosses the threshold of immune tolerance and develops a self-aimed immune response that gradually grows. This subclinical phase is manifested solely by the presence of antinuclear antibodies. The intensity of the autoimmune response increases until its products reach tissues and organs causing inflammation and clinical disease.

References

Bagavant H, Fu SM. Pathogenesis of kidney disease in systemic lupus erythematosus. Curr Opin Rheumatol. 2009;21(5):489–94.

Craft JE. Dissecting the immune cell mayhem that drives lupus pathogenesis. Sci Transl Med. 2011;3(73):73ps9.

Crispín JC, Kyttaris VC, Terhorst C, Tsokos GC. T cells as therapeutic targets in SLE. Nat Rev Rheumatol. 2010;6(6):317–25.
Diamond B, Volpe BT. A model for lupus brain disease. Immunol Rev. 2012;248(1):56–67.
Liu Z, Davidson A. Taming lupus-a new understanding of pathogenesis is leading to clinical advances. Nat Med. 2012;18(6):871–82.
Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol. 2001;1(2):147–53.
Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365(22):2110–21.

Systemic Lupus Erythematosus, Treatment

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Synonyms

Lupus treatment; Treatment of SLE

Definition

SLE is a systemic autoimmune disease that is treated with high-dose immunosuppressive medications during acute flares to prevent permanent organ damage. Less toxic medications are used during disease quiescence to prevent future flares. Besides the direct immunologic damage to organs and tissues, accelerated atherosclerosis and metabolic bone disease often accompany the disease and/or result from chronic use of toxic medications.

General Principals

Systemic Lupus Erythematosus (SLE) is characterized by periods of high disease activity (flares) and periods of clinical quiescence. The flares can be of varying severity, affect different organ systems, and can, if left untreated, lead to permanent organ damage (Tsokos 2011). SLE flares are treated with high-dose immunosuppressive medications, while less toxic immunomodulatory medications are used for flare prevention (most commonly used medications are described in Table 1). In addition, patients with SLE have oftentimes comorbid conditions, due to the disease itself and/or medications, that can result in significant disability.

Treatment of Acute SLE

SLE flares can be mild, moderate, or severe depending on the organs that are involved, the severity of inflammation, and the potential for permanent organ or tissue damage.

The treatment of severe organ or life-threatening SLE flares follows the paradigm established in patients with severe lupus nephritis (WHO class III and IV). Following the diagnosis of the type of lupus nephritis, treatment is started with high doses of corticosteroids (prednisone 1 mg/kg p.o. per day). If the disease is very active and especially if the kidney function is compromised, corticosteroids can initially be given intravenously at high doses (Methylprednisolone 1,000 mg IV per day for 3 days). Corticosteroids alone although efficacious have a suboptimal long-term effect with a significant proportion of the patients becoming hemodialysis dependent. Cyclophosphamide given monthly I.V. (0.5–1 mg/kg) when added to corticosteroids was found to be very effective in improving renal outcomes. More recently, mycophenolate mofetil, an oral immunosuppressive, was shown to be as good as cyclophosphamide in inducing remission of glomerulonephritis in SLE patients (reviewed in (Houssiau 2012)).

Initially cyclophosphamide treatment was given in two phases, a 6-month induction phase and a maintenance phase. Given significant toxicity of cyclophosphamide, other immunosuppressive medications are now routinely used instead of cyclophosphamide in the maintenance phase. Currently azathioprine (typically 2 mg/kg)

Systemic Lupus Erythematosus, Treatment, Table 1 Commonly used medications in SLE

Medication	Indication	Dose	Significant side effects
Hydroxychloroquine	Dermatitis and arthritis, pregnancy	200–400 mg/d po	Retinopathy (rare-yearly ophthalmologic exam); Muscle toxicity (very rare)
Azathioprine	Maintenance in lupus nephritis and other manifestations; can be used in pregnancy	50–150 mg (2 mg/kg) po	Liver and bone marrow toxicity
Prednisone	Acute flares; can be used in pregnancy	5 mg–60 mg po; IV bolus	Diabetes, hypertension; osteoporosis; weight gain; cataract; glaucoma
Cyclophosphamide	Lupus nephritis; other severe manifestations	0.5–1 mg/m2 IV q monthly	cytopenia; hemorrhagic cystitis; secondary cancers; infertility
Mycophenolate mofetil	Lupus nephritis; other manifestations	500–1,500 mg po bid	Diarrhea; cytopenia
Belimumab	mild-moderately active autoantibody positive SLE	10 mg/kg IV monthly	Infusion reactions; hypersensitivity; diarrhea
Methotrexate	arthritis	7.5–20 mg p.o. weekly	Bone marrow, liver, and lung toxicity; nausea, oral ulcers; folic acid can mitigate some of the side effects

or mycophenolate mofetil (2,000 mg per day) are the preferred maintenance treatment following the induction phase (current treatment guidelines published in (Bertsias et al. 2012)). Corticosteroids are used in a tapering fashion. The exact length of the maintenance phase of treatment is still unclear, but most experts agree that at least 2 years of treatment is required. These regimens coupled with better supportive care have improved the outcome of patients with nephritis from 90 % 5-year mortality to <10 % 10-year mortality.

By extrapolation from the nephritis trials, the combination of cyclophosphamide and corticosteroids is used also for the treatment of severe extrarenal flares as well. In particular multi-system disease, vasculitis, alveolar hemorrhage, and neuropsychiatric lupus are treated in the same fashion as nephritis with induction of remission using cyclophosphamide or mycophenolate together with corticosteroids followed by a maintenance phase. Class V nephritis, characterized by profound proteinuria without significant inflammation, is also generally treated with mycophenolate mofetil and varying doses of corticosteroids.

For less severe manifestations/flares of the disease such as pericarditis, pleuritis and, thrombocytopenia, the preferred treatment is moderate to high doses of corticosteroids (0.5–1 mg/kg of prednisone p.o.). Oftentimes an immunomodulator, such as azathioprine or mycophenolate mofetil, is added shortly after the initial phase to allow for a corticosteroid taper. The exact length of treatment with steroids, timing of introduction of an immunomodulator, and the length of the treatment are largely based on empiric data. Other modalities that can be used in SLE include intravenous immunoglobulin in patients with thrombocytopenia, dapsone and thalidomide for refractory skin disease, and plasmapheresis for alveolar hemorrhage.

Milder flares that cause organ inflammation but do not generally lead to organ damage are treated in a less aggressive way. Although corticosteroids are very effective in these cases, they should be avoided or used sparingly because of side effects. For arthritis and cutaneous disease the mainstay of treatment is the antimalarial drug hydroxychloroquine. Nonsteroidal anti-inflammatory drugs such as ibuprofen or diclofenac are also often used to alleviate symptoms of arthritis. In more severe cases of arthritis, methotrexate can be very effective. Skin rashes

often respond to local corticosteroids ointments; intra-lesional steroid injections and topical tacrolimus are used in more persistent skin rashes.

Flare Prevention

The most common readily recognizable cause of SLE flares is sun exposure. Ultraviolet light can cause skin rashes as well as constitutional symptoms in SLE patients. Strict sun protection is therefore part of the strategy to avoid SLE flares in most patients but especially in patients with known photosensitivity and/or skin disease. Physical or psychological stress, pregnancy, and viral illnesses may also precede an SLE flare although their cause-effect relationship is not established.

Despite preventive measures, a significant proportion of SLE patients flare at a high enough rate to require continuous treatment. As this treatment is chronic, less toxic medications are preferred. The most commonly used preventative medication in SLE is hydroxychloroquine, as it has a very good risk to benefit ratio.

If hydroxychloroquine does not prevent flares or the patient has continuous disease activity, immunosuppressive medications such as azathioprine, mycophenolate, or low-dose prednisone can be added to the regimen. As mentioned above, methotrexate can also be useful especially in patients with persistent or recurrent arthritis.

Prevention and Treatment of Comorbidities and Medication Toxicity

As is the case in several chronic inflammatory conditions, SLE patients display accelerated atherosclerosis, partly due to the disease itself and partly due to medication effects (Roman et al. 2003). Obesity, diabetes, and hypertension are common in SLE patients: Corticosteroids are the main but not the only contributors to these metabolic abnormalities; renal disease can cause hypertension and hypercholesterolemia; antiphospholipid antibodies can cause thromboembolic events. SLE patients should therefore be strongly encouraged to exercise regularly and avoid smoking. The blood pressure and the cholesterol level should be kept within normal limits and proteinuria should be addressed with the use of angiotensin converting enzyme inhibitors.

Corticosteroids are also largely responsible for osteopenia and/or osteoporosis in SLE patients. It is recommended that patients on chronic steroid treatment take supplemental calcium and vitamin D. Patients who have no contraindications to antiresorptive agents should also take bisphosphonate especially if they take more than 5–7.5 mg of prednisone a day.

Given that most medications used in SLE are immunosuppressive, the patients should be vaccinated against influenza yearly. Age-appropriate immunizations should also be done, although immunizations with live viruses should be avoided. SLE is associated with cancers especially lymphoma, while cyclophosphamide treatment predisposes to bladder cancer. Hence, periodic screening for development of symptoms and signs of malignancy, and age-appropriate malignancy laboratory screening including urinalysis for hematuria should be done in all SLE patients.

The patients should also be monitored for development of liver toxicity (mainly with methotrexate and azathioprine), bone marrow suppression (methotrexate, azathioprine, mycophenolate mofetil, and cyclophosphamide), and gastrointestinal complications such as mucosal or peptic ulcers, nausea, and diarrhea (nonsteroidal anti-inflammatories, prednisone, azathioprine, methotrexate, and mycophenolate mofetil). Yearly ophthalmologic exam is recommended to diagnose hydroxychloroquine maculopathy and screen for the development of cataracts and glaucoma in patients with high steroid exposure (Peponis et al. 2010).

As most SLE patients are women of childbearing years, several fertility issues arise with their treatment. Medications that can be used in patients that are pregnant or wish to become pregnant include hydroxychloroquine, prednisone, and azathioprine. Cyclophosphamide can cause infertility, which is more common in older women; concomitant use of leuprolide

may mitigate this risk. As estrogens have been associated with SLE, birth control pills have been thought as unsafe to use by SLE patients. Newer studies though have shown that they are probably safe to use in patients with low disease activity, but their safety during flares is still unclear (Sanchez-Guerrero et al. 2005). Use of birth control pills should also be avoided if the patient has high levels of antiphospholipid antibodies.

Emerging and Experimental Treatments

No new treatments had been approved for SLE for several decades until the approval of belimumab in 2011 (Furie et al. 2011). Belimumab is a monoclonal antibody that neutralizes the B Lymphocyte stimulator cytokine (BLyS), a pro-B cell growth factor that is thought to be important in the production of (auto)-antibodies in SLE. Following two large clinical trials, patients treated with monthly infusions of belimumab had less flares and needed less corticosteroids to manage their disease. Belimumab was only shown to be useful in patients with mild to moderate disease as patients with the more difficult to treat acute nephritis and central nervous system disease were excluded from the trials. Its effect overall on disease activity can be characterized as modest.

Difficult to treat SLE is not that uncommon. High-dose chemotherapy with or without stem cell transplantation can be helpful in patients with severe refractory SLE. Rituximab, an anti-CD20 antibody, can be helpful in select cases of SLE such as thrombotic thrombocytopenic purpura or immune thrombocytopenia. Thalidomide and dapsone are oftentimes used for severe skin disease. Calcineurin inhibitors are effective in SLE but due to unfavorable side effect profile especially renal toxicity are reserved as third or fourth line medications.

Several medications targeting B cells (epratuzumab, atacicept), T cells (abatacept), and soluble cytokines (tocilizumab) are being investigated for the treatment of SLE. Epratuzumab, an anti-CD22 antibody, showed some effect in phase II trials and is currently being investigated in phase III trials. Atacicept targets APRIL and BLyS, two B cell-related cytokines, caused severe infections when used in combination with mycophenolate. Abatacept is a fusion protein CTLA4-Ig that prevents T cell activation and although showing no effect in short-term trials, is being investigated in longer-term clinical trials.

Conclusions

The treatment of acute SLE consists of high doses of corticosteroids and immunosuppressives to prevent permanent organ damage. This initial induction of remission phase is followed by long-term maintenance treatment that aims at using the lowest possible dose of corticosteroids alongside an immunosuppressive medication. The choice of maintenance immunosuppressives depends on the severity of the disease and the organs involved. Hydroxychloroquine remains the drug of choice for prevention of future flares. Finally, although the monoclonal antibody belimumab was recently approved for the treatment of SLE, targeted SLE treatment remains in its infancy.

Cross-References

- ▶ Antiphospholipid Syndrome Treatment
- ▶ Novel Targets in Systemic Lupus Erythematosus
- ▶ Systemic Lupus Erythematosus, Clinical Features and Diagnosis
- ▶ Systemic Lupus Erythematosus, Clinical Trials
- ▶ Systemic Lupus Erythematosus, Pathogenesis

References

Bertsias GK, Tektonidou M, et al. Joint European league against rheumatism and European renal association-European dialysis and transplant association

(EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. Ann Rheum Dis. 2012;71:1771–82.

Furie R, Petri M, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. Arthritis Rheum. 2011;63(12):3918–30.

Houssiau FA. Therapy of lupus nephritis: lessons learned from clinical research and daily care of patients. Arthritis Res Ther. 2012;14(1):202.

Peponis V, Kyttaris VC, et al. Ocular side effects of anti-rheumatic medications: what a rheumatologist should know. Lupus. 2010;19(6):675–82.

Roman MJ, Shanker BA, et al. Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. N Engl J Med. 2003;349(25):2399–406.

Sanchez-Guerrero J, Uribe AG, et al. A trial of contraceptive methods in women with systemic lupus erythematosus. N Engl J Med. 2005;353(24):2539–49.

Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365(22):2110–21.

T

T Cell Memory

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Synonyms

Memory $CD8^+$ and $CD4^+$ T cells; Memory T lymphocytes

Definition

T cell memory arises from a complex molecular process in which a naïve T lymphocyte encounters its cognate antigen, expands in numbers, and acquires long-lived and functionally improved features. This process results in the most efficient reactivation of such T lymphocyte upon another encounter with the same antigen.

Introduction

Over the past decade, many studies have investigated the role of cells, pathways, and molecules that regulate this complex process. Differentiation of naïve T cells into fully functional long-lived memory T cells comprises several steps that include initial activation (priming), proliferation (expansion), and massive apoptosis (contraction), after which the cells that survived remain in the host and differentiate into long-lived memory cells (Cui and Kaech 2010; Harty and Badovinac 2008; McKinstry et al. 2010). Unraveling the molecular basis of T cell memory development is highly significant with regard to the development of novel vaccines and cell-based immunotherapies. The current state of knowledge on the mechanisms regulating the steps of T cell memory formation, maintenance, and protective abilities is summarized in this entry (Fig. 1).

Programming Naïve T Cells to Become Long-Lived Memory Cells

T cells that have successfully completed thymic selection exit to the blood and peripheral tissues and constitute the pool of host naïve T cells. Differentiation of such naïve, "antigen-inexperienced" T cells into long-lived "antigen-experienced" memory T cells is a complex multistep process that involves priming, expansion, contraction, and memory formation. During the first phase of this process, i.e., priming and proliferation, the majority of naïve T cells differentiate into effector cells while only a small proportion will become long-lived memory T cells.

T Cell Priming

The signals: Optimal T cell priming and differentiation into memory cells requires three

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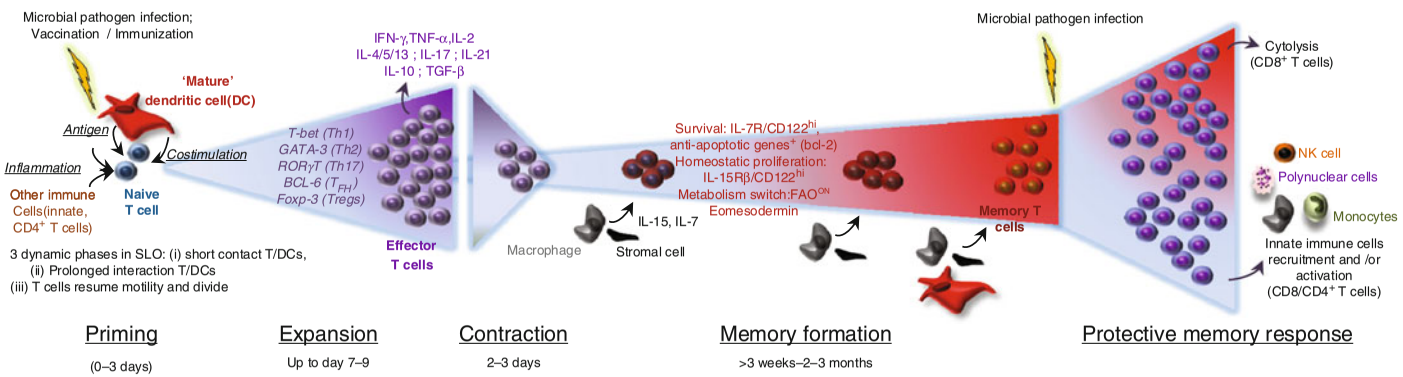

T Cell Memory, Fig. 1 Schematic representation of the steps controlling T cell memory formation and protective immune responses

signals, namely, antigen, costimulation, and inflammation. Each of these signals has an impact on T cell differentiation. Amount and duration of antigen presentation (signal 1) and subsequent T cell receptor (TCR) triggering largely determines the extent of T cell expansion. Costimulation (signal 2) that requires upregulation of cell-surface costimulatory molecules such as CD80 and CD86 on antigen presenting cells (APCs), which present T cell cognate antigens, is critical in driving potent T cell activation through engaging its major coreceptor CD28 on T cells. Finally, inflammatory factors (signal 3) such as IL-2, IL-12, IFN-γ, IL-4, IL-6, IL-10, TGF-β, or IFN-αβ, through signaling on the T cells, mostly modulate their ability to express specific sets of effector functions and to differentiate into distinct T cell subsets (Harty and Badovinac 2008; Littman and Rudensky 2010).

The cells providing the signals: Dendritic cells (DCs), a subset of mononuclear myeloid cells expressing high levels of the cell-surface integrin CD11c, are specialized APCs exhibiting the most potent ability to prime naïve T cells. Seminal reports established their unique functional features among the different subsets of APCs in efficient detection of microbial molecules, uptake, and presentation of antigens derived from microbial pathogens to T cells. Experiments were performed both in vitro and in vivo and compared the ability of the distinct APCs to activate naïve T cells. The most compelling evidence came from the generation of an in vivo conditional depletion system in which $CD11c^+$ cells expressed the human diphtheria toxin receptor (DTR), allowing for their selective elimination upon diphtheria toxin (DT) injection (Jung et al. 2002). Results demonstrated that DCs possess unique and optimized features for the priming of naïve T cells. Thus, upon recognition of pathogen-derived products through specialized family of innate immune receptors – namely, the pattern recognition receptors, PRRs – DCs undergo maturation and subsequently express high levels of major histocompatibility complex and of costimulatory molecules and secrete inflammatory cytokines, all of which are required for optimal T cell priming (Iwasaki and Medzhitov 2004). Other innate immune cells such as tissue-resident macrophages, blood phagocytes like neutrophils and monocytes that are rapidly recruited to infected foci, or even non-hematopoietic cells (stromal, epithelial, or endothelial cells) can also provide important inflammatory molecules at the time of T cell priming. Importantly, $CD4^+$ T cells, also defined as "helper" T cells (Th), are essential for optimal $CD8^+$ T cell priming and further differentiation because they contribute to DCs maturation, in particular through CD40 (on DCs)/CD40L (on $CD4^+$ T cells)-mediated costimulation. $CD4^+$ T cell help is particularly important for $CD8^+$ T cells primed in an environment with little inflammatory cytokines.

The dynamics of T cell priming in situ: Interaction of mature DCs with naïve T cells recognizing their cognate antigen takes place in secondary lymphoid organs (SLO), e.g., spleen and draining lymph nodes. Using intravital imaging by biphoton microscopy, the dynamics of DC-mediated T cell priming was visualized in living mice and revealed that naïve T cells and DCs presenting their cognate antigen encounter each other inside SLO by wandering in contact with a network of fibroblastic reticular cells (FRCs) and through complex and multiple chemotactic cues (Germain et al. 2012). The kinetics of naïve T cell priming involves three major dynamic phases in which T cells undergo intermittent contacts with cognate-antigen-loaded DCs, form stable interactions in clusters constituted of T cells surrounding individual DCs, and finally resume their motility prior to initiating multiple rounds of proliferation (Mempel et al. 2004).

T Cell Expansion, Effector Differentiation, and Contraction

The widely accepted view is that inflammation plays a key role in driving naïve T cell differentiation into highly effector cells that also exhibit a short-life potential and do not survive for extended period of times in the host. Effector T cells are characterized by upregulation of the

cell-surface lectin KLRG-1 and the IL-2 receptor CD25 as well as the loss of the chemokine receptor CCR7 and L-selectin CD62L (Cui and Kaech 2010; Sallusto et al. 1999). One important attribute of effector $CD8^+$ T cells is their ability to become cytolytic, express high amounts of granzyme B and perforin, and secrete inflammatory cytokines like IFN-γ and TNF-α.

In the case of effector $CD4^+$ T cells, a specific pattern of secreted cytokines is associated with distinct lineages of $CD4^+$ Th cells, such as Th1 cells producing IFN-γ/TNF-α/IL-2, Th2 cells producing IL-4/5/13 (Th2), Th17 cells producing IL-17/22, or follicular helper T cells (T_{FH}) producing IL-21 and regulating B cell effector responses. These effector cell types are usually associated with different diseases, in particular, strong Th1-type immune responses are promoted upon viral and intracellular bacterial infections; Th2-type immune responses are found upon parasitic worm infections or allergic diseases (e.g., asthma); and Th17-type immune responses are described during fungal and extracellular bacterial infection or autoimmune inflammatory diseases.

While the inflammatory context, e.g., which combination of cytokines are present at the time of initial T cell priming, is a critical inducer of T cell effector fate, expression of transcriptional regulators ultimately convey the priming signals and regulate the fates of the differentiating T cells. Some of the most important of these transcription factors controlling the differentiation of $CD8^+$ T cells are T-bet, eomesodermin (Eomes), Blimp-1, and BCL-6. Their roles have some redundancy (T-bet, Eomes) as well as clear antagonizing effects (Blimp-1, BCL-6) that regulate the balance between terminal effector and memory $CD8^+$ T cell fates in vivo (Crotty et al. 2010). T-bet but also the transcriptional regulators GATA-3, RORγT, or BCL-6 drive the differentiation of naïve $CD4^+$ T cells into distinct subsets of effector $CD4^+$ T cells, respectively, the Th1, Th2, Th17, and T_{FH} cells (Littman and Rudensky 2010).

After primary expansion, most T cells (>95 %) undergo programmed cell death. Current thinking is that T cell contraction is programmed early on during the initial priming. Inflammatory cytokines such as IFN-γ and IL-12 are strong contributors, possibly through the modulation of the level of anti-apoptotic molecules like BCL-2 inside T cells.

Memory Formation

Naïve T cells undergoing priming acquire the potential to differentiate into effector cells and memory cells. Adoptive transfers of one single naïve $CD8^+$ T cell of known antigenic specificity or its tracking through unique genetic barcoding indeed demonstrated that an individual $CD8^+$ T cell has the intrinsic potential to differentiate into various functional subsets of T cells following antigen-driven activation (Stemberger et al. 2007). One explanation supported by some reports is that T cells undergo asymmetric division during initial activation, leading to distinct effector or memory fates. Other elegant genetic tagging experiments in which activated $CD8^+$ T cells that express the cytolytic marker granzyme B became permanently labeled established that memory T cells may arise from effector cells (Jacob and Baltimore 1999).

Differentiation of fully functional long-lived memory $CD8^+$ T cells requires the presence of $CD4^+$ T cells, since memory $CD8^+$ T cells generated in the absence of $CD4^+$ T cells poorly re-expand upon recall stimulation. One mechanism accounting for this observation is that "helpless" memory $CD8^+$ T cells express increased amounts of proapoptotic molecules such as the TNF-related apoptosis-inducing ligand TRAIL (Cui and Kaech 2010; Harty and Badovinac 2008).

While strong inflammation during priming promotes T cell differentiation towards terminal effector cells, the nature of the infection will select for memory T cells with distinct functional features. For instance, during acute infections, in which microbial pathogens are eliminated from the host, memory cells come in at least two flavors, namely, effector (T_{EM}) and central (T_{CM}) memory T cells. Both subsets of memory T cells exhibit different properties: T_{CM} express CD62L and CCR7 for efficient trafficking to SLO and exhibit a high proliferative

capacity, while T_{EM} ($CD62L^{low}CCR7^{-}$) presence is largely restricted to nonlymphoid peripheral tissues in which they can express rapid effector functions contributing to early host protection (Sallusto et al. 1999). Expression of high levels of the cell-surface marker KLRG-1 on $CD8^+$ T cells further marks terminally differentiated "short-lived" effector cells (SLEC) within the T_{EM} subset, by opposition to $KLRG1^{low}$-activated T cells that give rise to long-lived memory cells (Cui and Kaech 2010). T_{CM} ultimately represent memory cells that persist in immunized hosts. While Th1- and Th2-committed $CD4^+$ T cells can form long-lived memory, it is less clear whether the other subsets of $CD4^+$ T cells (Th17, T_{FH}) per se persist in the host as true long-lived memory cells.

In contrast to acute infections, in the course of chronic infections, the fate of memory T cells is different. Seminal studies comparing two clonal variants of the lymphochoriomeningitis virus (LCMV) that either induce an acute or a chronic infection in mice established that repeated high level of antigenic stimulation of memory T cells in a low inflammatory context makes them dysfunctional and defined as "exhausted" memory T cells (Barber et al. 2006). These important findings were confirmed in human immunodeficiency and hepatitis B and C virus-infected patients. Constantly, TCR-triggered memory T cells express very high levels of inhibitory receptors such as PD1, one of the main markers characterizing T cell exhaustion.

Mechanisms of Long-Term Memory T Cell Maintenance

Memory T cells can persist in the host for very long period of time, and these unique features are achieved through several mechanisms that are discussed below.

The cytokines IL-7 and IL-15: These cytokines are among the most important contributors to memory T cell maintenance with IL-7 and IL-15 respectively involved in survival and homeostatic turnover (Surh and Sprent 2008). In the course of a primary microbial pathogen infection, while the majority of activated effector T cells downregulate cell-surface expression of the IL-7 receptor (IL-7R/CD127), a small subset expresses high cell-surface levels. Adoptive transfer experiments of this subset of cells into a recipient host demonstrated that they differentiated into long-lived memory cells; however, IL-7R expression per se is not instructive of the process. Since IL-7 is constitutively secreted at relatively fixed amounts by FRCs in SLO, modulation of IL-7 levels remains limited, suggesting that regulation of IL-7 signals may mostly occur through fine-tuning of cell-surface IL-7R expression by the lymphocytes. Downstream effectors of IL-7 signaling inside memory T cells include anti-apoptotic molecules such as Bcl-2 which undergo upregulation. IL-15 plays a critical role for memory T cell maintenance. IL-15 mostly acts by promoting homeostatic proliferation of the memory T cells that express high cell-surface levels of the IL-15Rβ chain (CD122). Upon adoptive transfer into $IL\text{-}15^{-/-}$ recipients, memory T cells are rapidly lost, demonstrating IL-15 signals absolute requirements for memory T cell maintenance. Reports have also shown that IL-15 is *trans*-presented by distinct APCs – namely, DCs and macrophages (MPs) – to $CD8^+$ T cells, and the different APCs exhibit specific roles: MPs support effector $CD8^+$ T cell transition to the memory stage and T_{EM}/T_{CM} homeostasis, while DC-derived IL-15 appears restricted to T_{CM} (Mortier et al. 2009). Of note, the long-term maintenance of memory $CD8^+$ T cells induced after acute infections requires the presence of $CD4^+$ T cells even though the mechanisms involved still need to be clarified.

Several transcription factors involved in T cell development ultimately regulate the maintenance and reactivation of memory T cells. These include transcriptional regulators such as BCL-6, the T cell factor 1 (TCF-1), or the inhibitor of DNA-binding 2 (Id2). Common mechanisms shared by these factors include promoting the expression of anti-apoptotic molecules like BCL-2 and the IL-15 receptor CD122 involved in homeostatic proliferation.

Metabolic switch: T cells undergoing priming, expansion, and memory differentiation are experiencing dramatic energetic changes to accommodate their rapidly evolving status. Recent reports revealed that upon differentiation into memory T cells, expanding T cells switch from an anabolic (glycolysis) to a catabolic metabolism by inducing fatty acid oxidation (FAO) (Pearce et al. 2009). This process is required for their long-term maintenance and also suggested to depend on IL-15 and on the transcription factor eomesodermin. The use of FAO as the main source of energy correlates with acquisition of resting quiescent features by the memory T cells.

Antigen and major histocompatibility complex molecules: While naïve T cells require frequent contacts with major histocompatibility complex molecules and endogenous peptides, memory T cells can persist in the host for very long period of time in the absence of major histocompatibility complex presented cognate antigens. This idea was supported by adoptive transfer experiments of memory T cells purified from immunized hosts to mice genetically deficient for major histocompatibility complex class I or class II molecules in comparison to wt mice, and the numbers of memory cells was shown to remain comparable. This represents another important adaptative feature of memory T cells.

Mechanisms of Protection

T cell memory is an essential part of the adaptive immune system of the host. It allows hosts to most efficiently resist to microbial pathogens against which they have been previously immunized. Several mechanisms account for this: memory T cells specific for an immunizing antigen are present in much higher numbers than their naïve counterparts (up to 200 times); they exhibit distinct activation thresholds and requirements and acquire enhanced effector functions and improved abilities to be mobilized during inflammation.

Memory T cells are present in much higher numbers than their naïve counterparts in immunized hosts as a consequence of their initial clonal expansion but also because they survive for extended times through expression of anti-apoptotic genes, homeostatic proliferation and metabolism switch, and loss of major histocompatibility complex antigen dependence. They also constitutively express "ready-to-go" cell-cycle-related kinases and effector cytokines and chemokines stored as mRNA and proteins, which make them able not only to rapidly initiate proliferation but also to deliver protective effector functions (Veiga-Fernandes et al. 2000). Importantly, memory T cells express increased levels of chemotactic receptors and adhesion molecules that are essential to efficiently reach infected foci and mediate host defense (Sallusto et al. 2000).

Immunological protection, e.g., microbial pathogen killing, can be achieved through several mechanisms. Memory $CD8^+$ T lymphocytes recognize and kill infected or tumor cells through delivery of cytotoxic granules into target cells and/or triggering of cell-surface death receptors. Memory $CD4^+$ T lymphocytes are best characterized as important sources of effector cytokines and chemokines that promote both activation and recruitment of antimicrobial innate immune effector cells (McKinstry et al. 2010). Such mechanism, which can also be achieved by memory $CD8^+$ T cells in some instances (Narni-Mancinelli et al. 2007), allow for rapid and efficient amplification of the host-protective immune response. Along these lines, the concomitant expression of multiple effector functions by memory T cells is strongly correlated to protection both in humans and in mice models (Seder et al. 2008). Successive immunizations with a same vaccine promote the acquisition of a core genetic program by memory cells that includes high transcription of effector and chemokine genes and receptors, as well as anti-apoptotic genes (Cui and Kaech 2010). Interestingly, in hosts sequentially infected with multiple microbial pathogens, the global size of the memory T ($CD8^+$) compartment increases while only a minimal loss of pathogen-specific memory cells was observed. Overall, while many distinct processes exposed above account for heightened

memory T cell-mediated host protection, achieving multifunctional potential is likely the most relevant to hosts' survival.

Conclusions

While tremendous insights have been gained in the complex process of T cell memory formation over the past decade, the fine understanding on how these events take place in situ in a living host and the cell-cell interactions and molecular cues that govern these processes in a timely manner are still poorly defined. Whether a specific set of innate immune sensing receptors inside APCs drives the differentiation of memory T cells at the time of priming is an open possibility. Likewise, which combinations of signals lead to the differentiation of memory T cells acquiring specific functions and the complex interaction of transcription factors regulating these processes needs to be precisely determined so that the design of T cell-dependent vaccination and therapeutic strategies will be rationally improved.

Cross-References

- B7 and CD28 Families
- Bcl-2 Family Members and Lymphocyte Homeostasis
- CD40
- Chemokines
- Cytotoxic T Lymphocytes
- Mammalian Target of Rapamycin (mTOR)

References

Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439:682–7.

Crotty S, Johnston RJ, Schoenberger SP. Effectors and memories: Bcl-6 and blimp-1 in T and B lymphocyte differentiation. Nat Immunol. 2010;11:114–20.

Cui W, Kaech SM. Generation of effector CD8+ T cells and their conversion to memory T cells. Immunol Rev. 2010;236:151–66.

Germain RN, Robey EA, Cahalan MD. A decade of imaging cellular motility and interaction dynamics in the immune system. Science. 2012;336:1676–81.

Harty JT, Badovinac VP. Shaping and reshaping CD8+ T-cell memory. Nat Rev Immunol. 2008;8:107–19.

Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol. 2004;5:987–95.

Jacob J, Baltimore D. Modelling T-cell memory by genetic marking of memory T cells in vivo. Nature. 1999;399:593–7.

Jung S, Unutmaz D, Wong P, Sano G, De los Santos K, Sparwasser T, Wu S, Vuthoori S, Ko K, Zavala F, et al. In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. Immunity. 2002;17:211–20.

Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. Cell. 2010;140:845–58.

McKinstry KK, Strutt TM, Swain SL. The potential of CD4 T-cell memory. Immunology. 2010;130:1–9.

Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature. 2004;427:154–9.

Mortier E, Advincula R, Kim L, Chmura S, Barrera J, Reizis B, Malynn BA, Ma A. Macrophage- and dendritic-cell-derived interleukin-15 receptor alpha supports homeostasis of distinct CD8+ T cell subsets. Immunity. 2009;31:811–22.

Narni-Mancinelli E, Campisi L, Bassand D, Cazareth J, Gounon P, Glaichenhaus N, Lauvau G. Memory CD8+ T cells mediate antibacterial immunity via CCL3 activation of TNF/ROI + phagocytes. J Exp Med. 2007;204:2075–87.

Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG, Choi Y. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature. 2009;460:103–7.

Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999;401:708–12.

Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol. 2000;18:593–620.

Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nat Rev Immunol. 2008;8:247–58.

Stemberger C, Huster KM, Koffler M, Anderl F, Schiemann M, Wagner H, Busch DH. A single naive CD8+ T cell precursor can develop into diverse effector and memory subsets. Immunity. 2007;27:985–97.

Surh CD, Sprent J. Homeostasis of naive and memory T cells. Immunity. 2008;29:848–62.

Veiga-Fernandes H, Walter U, Bourgeois C, McLean A, Rocha B. Response of naive and memory CD8+ T cells to antigen stimulation in vivo. Nat Immunol. 2000;1:47–53.

TGF-β

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Synonyms

Transforming growth factor-beta (TGF-β)

Definition

TGF-β is a dimeric polypeptide growth factor which belongs to a family of growth factors that share a cluster of conserved cysteine residues held together by intramolecular disulfide bonds (Elgert 2009a).

It is produced by cells which express receptors for it, which include most human cells.

TGF-β regulates many biological functions including the proliferation and differentiation of cells, embryonic development, wound healing, and angiogenesis (Elgert 2009a).

Mechanism of Action

TGF-β exists in three isoforms: TGF-β1, TGF-β2, and TGF-β3. Each isoform is encoded by a distinct gene and is expressed in a tissue-specific manner. TGF-β1 is expressed mainly in endothelial, hematopoietic, and connective tissue cells. TGF-β2 is produced in epithelial and neuronal cells, and TGF-β3 is produced mainly in mesenchymal cells. All three isoforms are highly conserved in mammals, suggesting a critical biological function for each one (Elgert 2009a; Santibanez et al. 2011).

To regulate cellular processes, TGF-β binds three high-affinity cell-surface receptors known as TGF-β receptor I, II, and III. The TGF-β type III receptor is the most abundant (Elgert 2009a).

After binding the specific receptor, TGF-β intracellular signaling occurs predominantly by phosphorylation of cytoplasmic proteins belonging to the Smad family. The last Smad-phosphorylated complex is shuttled into the nucleus where it regulates the expression of target genes by direct binding to the gene promoter and/or through an interaction with other transcriptional factors (Liu and Feng 2010). In addition to the canonical Smad pathway, TGF-β can also regulate cellular processes through Smad-independent pathways that include the activation of other signaling molecules such as mitogen-activated protein kinase (MAPK), Akt, or nuclear factor κB (Santibanez et al. 2011; Liu and Feng 2010).

Role in Immunity

TGF-β is produced by all leukocytes, promoting their differentiation and inhibiting their proliferation and activation. Furthermore, TGF-β provides leukocyte chemotaxis by regulating expression of adhesion molecules (Elgert 2009a; Santibanez et al. 2011).

Although TGF-β had been classified as an immunosuppressive cytokine by inhibiting T lymphocyte activation, recent observations indicate that the proinflammatory cytokine interleukin-6 switches the transcriptional program initiated by TGF-β, inducing development of Th17-activated lymphocytes (Miossec et al. 2009). Th17 cells can rapidly initiate an inflammatory response and facilitate clearance of infections. In addition, unregulated Th17 responses or overproduction of interleukin-17 is associated with chronic inflammation as well as autoimmunity and autoinflammatory conditions (Miossec et al. 2009).

Role in Cancer

TGF-β regulates cellular proliferation in a cell-specific manner. In most epithelial, endothelial, and hematopoietic cells, it is a potent inhibitor of cell proliferation, arresting the cell

cycle in the G1 phase. Thus, in normal conditions TGF-β has a tumor-suppressive role (Elgert 2009b; Finn 2008). In addition, TGF-β is able to protect cells from tumorigenesis by inducing apoptosis (programmed cell death) and by maintaining genomic stability (inhibiting the production of mitogenic factors) (Finn 2008).

Paradoxically, TGF-β becomes a pro-oncogenic factor in cancer cells in which mutations in TGF-β signaling confer resistance to the tumor-suppressive role of TGF-β. Resistance to TGF-β cell cycle control allows cancer cells and surrounding stromal cells (fibroblasts) to proliferate and in turn to produce more TGF-β. The overproduction of TGF-β leads to immunosuppression and angiogenesis and increases the invasiveness of the tumor by activating fibroblasts, immune cells, and endothelial and smooth-muscle cells (Elgert 2009b; Finn 2008). By suppressing activation of infiltrating immune cells, TGF-β allows tumor cells to escape host immunosurveillance. In addition, with the induction of angiogenesis, TGF-β stimulates growth of late-stage tumors, thereby facilitating invasion and metastasis (also enhanced by TGF-β-mediated cell motility) (Finn 2008; Chiang and Massague 2008).

Role in Fibrosis

TGF-β is one of the most potent regulators of the production and deposition of extracellular matrix (ECM). It stimulates fibroblasts and other cells to produce ECM proteins and cell-adhesion proteins, including collagen, fibronectin, and integrins. In addition, TGF-β reduces production of enzymes that degrade the ECM, including collagenase, heparinase and stromelysin, and increases production of proteins that inhibit ECM degrading enzymes (Smith 2003; Border and Noble 1994; Gabrielli et al. 2009).

Although TGF-β is essential for wound healing, overproduction of TGF-β can result in excessive deposition of scar tissue and fibrosis. Thus, abnormal TGF-β signaling results in fibrotic diseases such as pulmonary fibrosis, liver cirrhosis, and systemic sclerosis. In these pathologies, the aberrant upregulation of TGF-β in an autocrine/paracrine manner leads to a constitutive activation of fibrogenesis. Together with other profibrotic cytokines, TGF-β orchestrates excessive ECM synthesis and deposition, thereby promoting early fibrosis without normal remodeling of tissue, a process that is subsequently maintained by other profibrogenic cytokines, including connective-tissue growth factor (CTGF) (Border and Noble 1994; Gabrielli et al. 2009; Mori et al. 1999).

Cross-References

- ▶ Fibrosis
- ▶ Scleroderma (Systemic Sclerosis): Pathogenesis and Clinical Manifestations
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. N Engl J Med. 1994;331:1286–92.

Chiang AC, Massague J. Molecular basis of metastasis. N Engl J Med. 2008;359:2814–23.

Elgert KD. Cytokines. In: Immunology: understanding the immune system. New York: Wiley; 2009a. p. 285–320.

Elgert KD. Tumor immunology. In: Immunology: understanding the immune system. New York: Wiley; 2009b. p. 489–528.

Finn OJ. Cancer immunology. N Engl J Med. 2008;358:2704–15.

Gabrielli A, Avvedimento EV, et al. Scleroderma. N Engl J Med. 2009;360:1989–2003.

Liu T, Feng XH. Regulation of TGF-beta signalling by protein phosphatases. Biochem J. 2010;430:191–8.

Miossec P, Korn T, et al. Interleukin-17 and type 17 helper T Cells. N Engl J Med. 2009;361:888–98.

Mori T, Kawara S, et al. Role and interaction of connective tissue growth factor with transforming growth factor-beta in persistent fibrosis: a mouse fibrosis model. J Cell Physiol. 1999;181:153–9.

Santibanez JF, Quintanilla M, et al. TGF-β/TGF-β receptor system and its role in physiological and pathological conditions. Clin Sci. 2011;121:233–51.

Smith EA. Systemic sclerosis: etiology and pathogenesis. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Wisman MH, editors. Rheumatology. Edinburgh: Mosby; 2003. p. 1481–92.

Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

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Definition

The emerging area of renal clinical pharmacology has uncovered the complicated nature of drug selection and dosing in chronic kidney diseases. This is particularly relevant to optimizing treatment-related outcomes for patients with glomerulonephritis. The pharmacokinetics and pharmacodynamics of drugs need to be fully understood in order to adequately dose and assess the therapeutic benefits of existing and novel treatments.

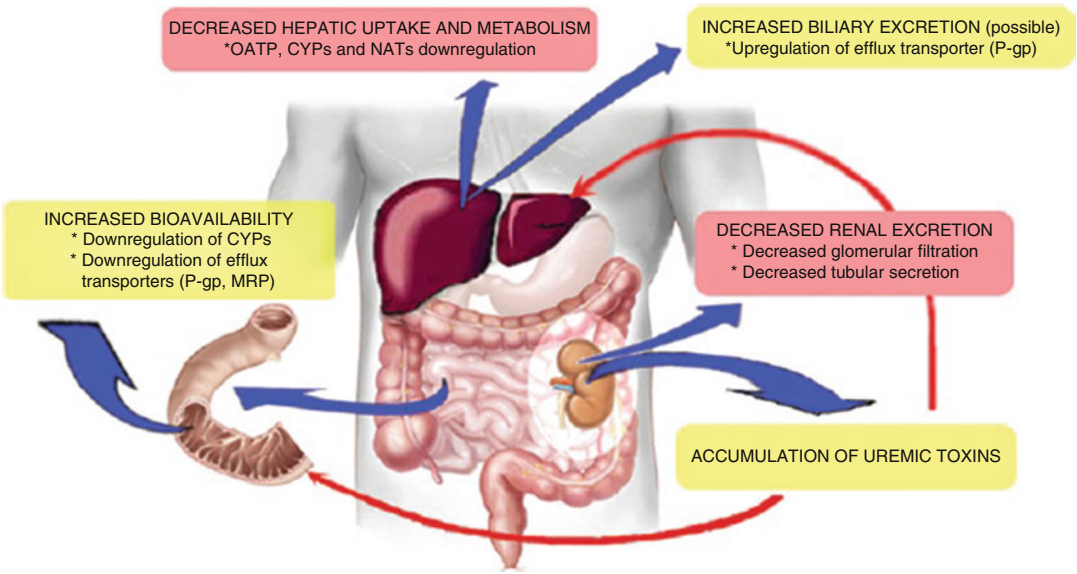
Background

In 2010, more than 20 million people in the USA aged 20 years and older were reported to have chronic kidney disease (NKUDIC 2011). This statistic represents greater than 10 % of the entire population. Diabetes mellitus is the leading cause of kidney disease in the United States, and with its rising incidence, there is a corresponding rise in diabetic kidney disease. Similar associations with kidney disease are also anticipated with the rising incidence and prevalence of hypertension, obesity, hyperlipidemia, and aging in the US population.

The US trends between primary diseases and kidney disease are mirrored on a global level. Research throughout the world supports a high prevalence of kidney disease (Chadban et al. 2003; Jafar et al. 2005; Choi et al. 2006). A study from Korea, using microalbuminuria as a marker for kidney disease, found a prevalence of microalbuminuria of 2.8 %, 10.1 %, and 16.0 % in normoglycemic, normotensive patients; hypertensive patients; and diabetic patients, respectively (Choi et al. 2006). The AusDiab Kidney Study found that approximately 16 % of the adult Australian population has some degree of kidney injury, as defined by proteinuria, hematuria, and/or glomerular filtration rate (GFR) (Chadban et al. 2003). A small study in Pakistani patients designed to evaluate the accuracy of commonly used equations for estimating GFR found that 26.7 % of men and 32.5 % of women (overall prevalence 29.9 %) age 40 years and older had a reduced GFR (Jafar et al. 2005). The global trends in increasing prevalence rates of kidney disease necessitate a thorough understanding of therapeutic interventions to slow disease progression, as well as improvements in methods of treating concomitant comorbidities.

Therapeutic Considerations in Kidney Diseases

Therapeutics are complicated in kidney diseases (Fig. 1). Typical reasons for this include reductions in glomerular filtration rate, loss of proteins in the urine, and alterations in cellular components of blood, serum proteins, and electrolytes. Many medications or their metabolites are dependent on the kidneys for elimination from the body. There is a highly correlated relationship between glomerular filtration rate and drug dosage and/or administration frequency requirements. Historical published data from the ESRD population show altered interactions between drugs and plasma proteins. This is particularly relevant for patients receiving drugs that exhibit significant protein-binding characteristics. A classic example is phenytoin, where there is an elevated free fraction of the drug in patients with ESRD. Some data also support enhanced clearance of highly protein-bound drugs in patients with glomerulonephritis. Baseline alterations in electrolytes due to chronic kidney disease can influence the toxicity profile of many medications. Additional emerging data demonstrate changes in the activity



Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis, Fig. 1 Potential mechanisms for altered drug disposition in kidney diseases (Nolin et al. 2008)

of drug metabolism and drug transport pathways in patients with chronic kidney diseases (Nolin et al. 2008; Joy et al. 2012) (Fig. 1). The existing data support reductions in activity in many drug metabolism pathways in primary and secondary kidney diseases. These established and evolving clinical paradigms demonstrate the potential for significant inaccuracies in therapeutic dosing regimens in chronic kidney disease patients. The existing and emerging awareness of the complicated nature of drug selection and dosing in chronic kidney disease provides evidence that clinicians must increase their understanding of clinical pharmacology in the context of kidney disease.

The etiology of the kidney disease needs to be considered in order to predict the physiological aspects that may contribute to altered disposition of drugs. Patients with glomerulonephritis can have significant perturbations in serum albumin secondary to loss of proteins through the urine. They can also exhibit loss of protein-bound drug in the urine. Many of these patients have reductions in GFR as well. The relationship between drug exposure and pharmacodynamic response in patients with kidney disease can be confounded by whether the disease etiology is primary or secondary (Joy et al. 2009a). Further complicating drug therapy, disparate patterns of kidney injury exist, and variable rates of progression within different compartments of the kidney may occur depending, at least in part, on the etiology of disease. Inconsistency between methods of determining and calculating renal function may lead to differences in drug dosing and, therefore, response. The primary focus of this chapter is on therapeutic considerations specific to the pharmacokinetics and pharmacodynamics of drugs used in patients with glomerular diseases.

Glomerular Diseases and Treatment Considerations

The primary glomerular diseases represent the third leading cause of end-stage renal disease in the United States. This group of diseases (Table 1) comprises several entities, such as IgA nephropathy, focal segmental glomerular sclerosis (FSGS), lupus nephritis, and idiopathic membranous nephropathy. Since some causes of vasculitis also lead to glomerulonephritis, they are included in Table 1.

Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis, Table 1 Causes of glomerular disease (Hunder 2013; Hebert 2012)

IgA nephropathy
Thin basement membrane disease
Hereditary nephritis
Mesangial proliferative glomerulonephritis
Systemic lupus erythematosus
Post-infectious glomerulonephritis
Membranoproliferative glomerulonephritis
Rapidly progressive glomerulonephritis
Fibrillary glomerulonephritis
Minimal change disease
Focal glomerulosclerosis
Membranous nephropathy
Diabetic nephropathy
Primary amyloidosis/light chain deposition disease
Benign nephrosclerosis
Vasculitis
Churg-Strauss syndrome (also known as allergic granulomatosis and angiitis)
Granulomatosis with polyangiitis (formerly known as Wegener's disease)
Microscopic polyarteritis
Anti-glomerular basement membrane (anti-GBM) disease
Henoch-Schönlein purpura
Essential cryoglobulinemic vasculitis
Hypersensitivity vasculitis
Vasculitis secondary to connective tissue disorders
Vasculitis secondary to viral infection

Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis, Table 2 Drugs used in the treatment of glomerulonephritis

Glucocorticoids
Solumedrol
Prednisone
Immunosuppressants
Mycophenolate mofetil
Cyclosporine
Tacrolimus
Cytotoxic agents
Cyclophosphamide
Azathioprine
Methotrexate
Chlorambucil
Therapeutic antibodies
Rituximab (anti-CD20)
Belimumab (anti-BLyS)

Many of the drugs currently used for the treatment of glomerular diseases (Table 2) were originally approved to treat rheumatologic diseases, malignancies, or prevention of rejection in transplant patients. Therapeutic dosing regimens were initially selected based on the ranges of doses used for the treatment of the approved indications. Subsequent modifications in dosing regimens have come about through clinical trials that have endpoints of efficacy and/or toxicity. A limitation to repurposing medications to new populations is the lack of understanding of the clinical pharmacology of the therapeutic agents in those populations. This is particularly relevant in patients with chronic kidney disease and, more specifically, in patients with glomerulonephritis. In fact, increased systemic clearance of these drugs has been observed in the setting of glomerulonephritis compared with reference diseases (Joy 2011; Joy et al. 2009b, c, d; Joy et al. 2010a). The enhanced systemic clearance has been observed for both small molecules and therapeutic proteins. As clearance is subdivided into "renal clearance" and "nonrenal clearance" fractions, it is important to understand the relevance of each clearance route on overall systemic clearance. Interestingly, both renal and nonrenal clearance enhancements have been reported in kidney diseases due to glomerulonephritis. Enhancements in renal clearance can occur secondary to increases in the activity of apical drug efflux transporters on proximal tubule cells and secondary to loss of protein-bound drugs in cases of proteinuria. Since eGFR is usually negatively impacted by glomerulonephritis, augmented renal clearance cannot be attributed to increases in eGFR. Enhancements in nonrenal clearance can occur secondary to increases in free fraction of drugs, resulting in an increased propensity to undergo drug metabolism, and may occur secondary to enhancement of certain drug metabolism pathways. The role of systemic inflammation on renal and nonrenal clearance pathways in glomerulonephritis has not been evaluated.

Additionally, the contribution of edema in patients with massive urinary protein excretion to a medication's volume of distribution and clearance has not been elucidated. Understanding alterations in the pharmacokinetics and pharmacodynamics of drugs in glomerulonephritis is central to personalizing patient therapies based on acceptable therapeutic targets. Smarter prescribing should result in better treatment-related outcomes across patient populations. A thorough understanding of clinical pharmacology in kidney diseases should reduce the potential for adverse drug effects and drug interactions. The following sections of this chapter provide information concerning the existing published pharmacology data on several medications commonly used in the treatment of glomerulonephritis.

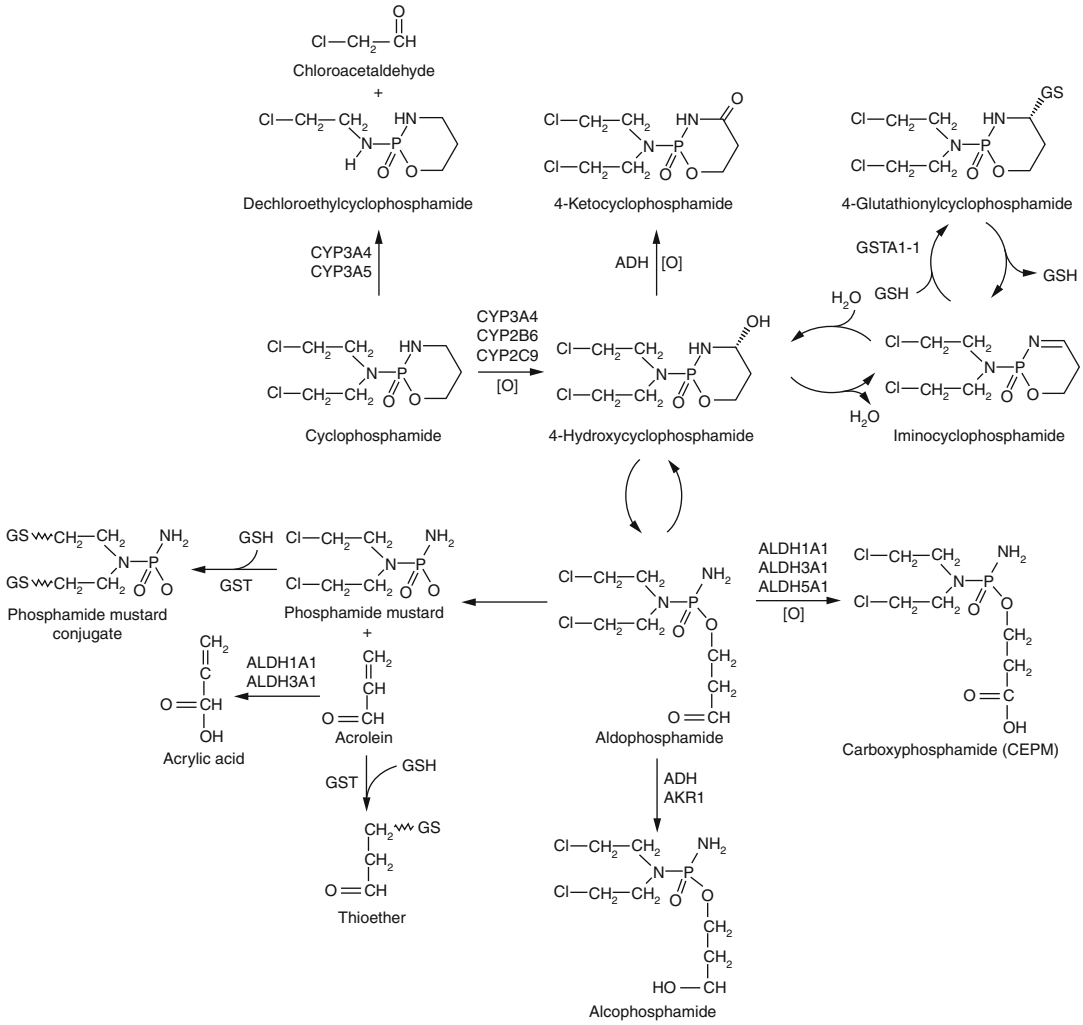
Treatments of Glomerulonephritis

Cyclophosphamide

Cyclophosphamide is used in the treatment of rapidly progressive forms of glomerulonephritis, including forms of small-vessel vasculitis (SVV) and lupus nephritis. One of the first accounts of cytotoxic therapy in the treatment of granulomatosis with polyangiitis (GPA; formerly Wegener's granulomatosis) was by Fahey et al. in the early 1950s (Fahey et al. 1954). The report was published in a 1954 volume of the *Lancet* and described a 38-year-old man with GPA who was treated with nitrogen mustard. Since that time, a number of cytotoxic therapies, including cyclophosphamide, azathioprine, chlorambucil, and methotrexate, have been used in the treatment of various immune-mediated diseases. Cyclophosphamide has remained the mainstay of treatment of many forms of glomerulonephritis. Previous studies in animals have demonstrated cyclophosphamide's immunosuppressant activity, independent of its antimitotic (i.e., cytotoxic) characteristics in the treatment of malignancy.

Cyclophosphamide is an oxazaphosphorine that is related to the nitrogen mustard family of alkylating agents. Cyclophosphamide is a prodrug that undergoes complex metabolic alterations in the liver and other tissues via multiple enzymatic pathways, including cytochrome P450s, aldehyde dehydrogenases, alcohol dehydrogenases, and glutathione sulfotransferases (Fig. 2). Of the multiple metabolites generated from the cyclophosphamide prodrug, 4-hydroxycyclophosphamide (4-OH-CPA) is the active metabolite that can be readily measured in plasma. It is in equilibrium with its tautomer, aldophosphamide, the aldehyde intermediate between 4-OH-CPA and the ultimate cytotoxic metabolite, phosphoramide mustard. When cyclophosphamide is used as an antitumor agent, phosphoramide mustard is thought to be the primary active antitumor metabolite, mediating tumor destruction by alkylation and resultant cross-linking of DNA in tumor cells. The 4-OH-CPA metabolite can also undergo further metabolism by aldehyde dehydrogenase to carboxyphosphamide (CEPM), which is nontoxic (Fig. 2). Studies in patients with severe aplastic anemia revealed that cells with high levels of aldehyde dehydrogenase, such as early hematopoietic progenitors, are comparatively resistant to cyclophosphamide. Alternatively, lymphocytes, which are relatively deficient in aldehyde dehydrogenase, are highly susceptible to cyclophosphamide, contributing to cyclophosphamide's effectiveness as an immunosuppressant.

Although cyclophosphamide has proven to be useful in the treatment of glomerulonephritis due to both SVV and systemic lupus erythematosus (SLE), treatment response remains variable between patients. For example, African-American race has been shown to be an independent predictor of poor outcome (i.e., decreased renal survival) in patients with lupus nephritis (Dooley et al. 1997). A clear interpretation of treatment responses had until the time of this study been confounded by the designs of previous trials. The earlier studies included predominantly Caucasian patients and did not distinguish among histological types of renal lesions. The more recent study by Dooley et al. demonstrated that renal survival in African-Americans with diffuse proliferative glomerulonephritis was markedly worse than in non-African-American patients with the same lesion. All of the patients



Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis, Fig. 2 Metabolic scheme for cyclophosphamide (Drug Metabolism Reviews (was Fig. 3 in 2005))

in the study were being treated with corticosteroids, with similar tapering schedules in African-Americans and non-African-Americans. Most patients were continued on steroid therapy for greater than 12 months. The study utilized modified NIH criteria for dosing of cyclophosphamide in lupus nephritis. Patients were treated with 0.5–1.0 g/m^2 cyclophosphamide once a month for 6 months, with dose adjusted to patients' nadir white blood cell counts. Quarterly cyclophosphamide infusions were continued dependent on the renal response of each patient. Overall, the median cumulative dose of intravenous cyclophosphamide was lower in African-Americans than in non-African-Americans due to a higher incidence of ESRD in African-Americans in the 6 months after kidney biopsy. Otherwise, there was no difference in dose or duration of treatment in patients who did not progress to ESRD within the first 6 months. The reported 58 % 5-year renal survival among African-Americans demonstrates that, even among patients who are ostensibly experiencing the same clinical condition and undergoing the same treatment, the outcome can be quite different. The complex

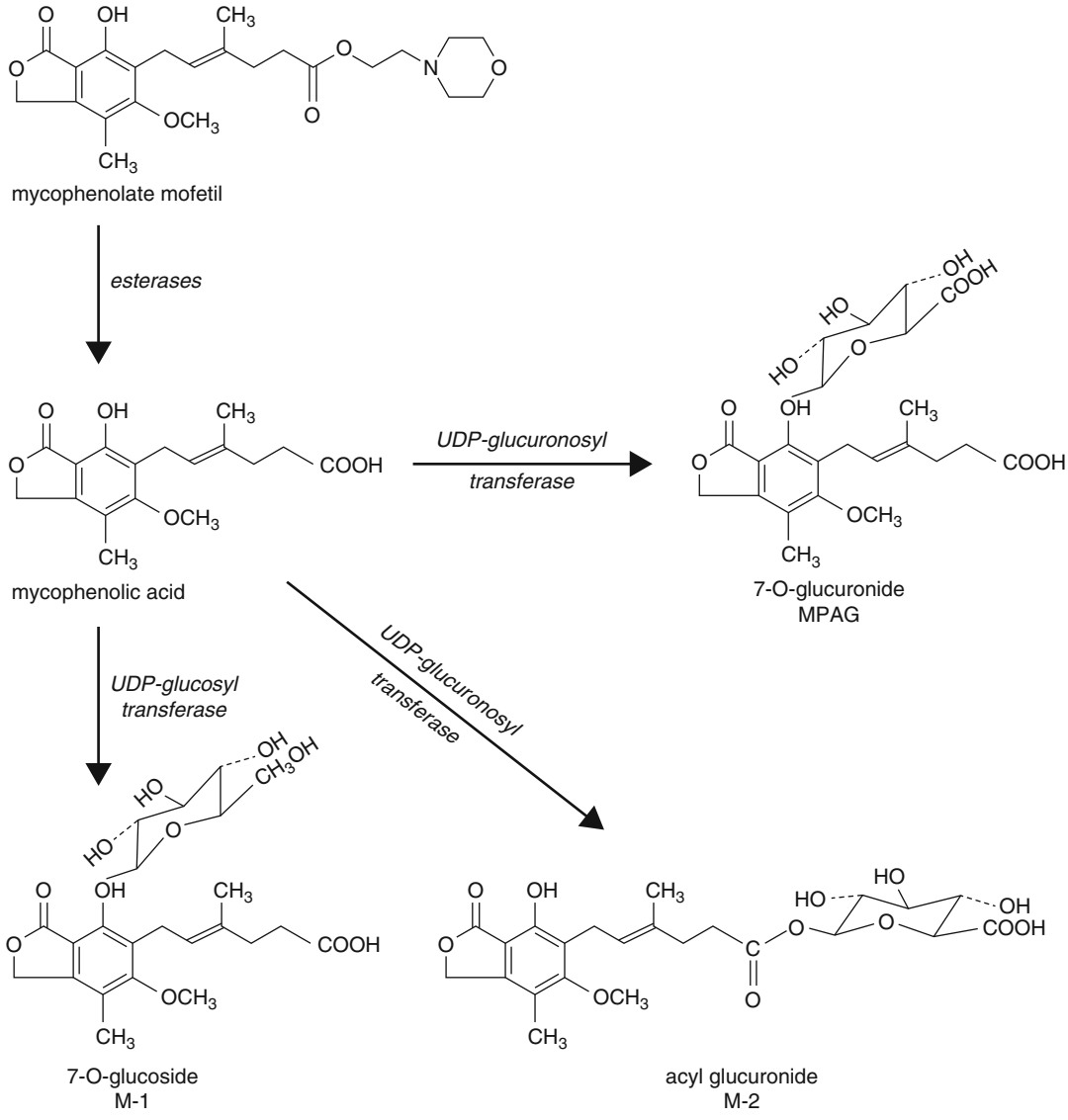
routes of metabolism and the pharmacokinetic variability of cyclophosphamide in these patients must be considered as a possible underlying source of this variability in treatment-related outcomes.

The influence of eGFR on the pharmacokinetics of cyclophosphamide has been reported (Haubitz et al. 2002). As compared to controls with eGFR ≥80 mL/min, patients with eGFR of 25–50 mL/min and 10–24 mL/min had total clearance and renal clearance values that were significantly reduced. These reductions resulted in cyclophosphamide exposures that were nearly double the values found in patients with eGFR ≥80 mL/min. Subsequent studies have reported on the pharmacokinetics of cyclophosphamide and 4-OH-CPA in patients with lupus nephritis and SVV (Belfayol-Pisanté et al. 2000; de Miranda Silva et al. 2009; Joy et al. 2012). In the Joy et al. study, the glomerulonephritis patients had relatively preserved eGFR, so the study focused on the influence of clinical factors such as serum albumin and urinary protein excretion on pharmacokinetics (Joy et al. 2012). Reductions in serum albumin resulted in reduced exposure to 4-OH-CPA. Additionally, urinary protein loss resulted in higher exposure to cyclophosphamide as compared to 4-OH-CPA. Refinement of the established relationships between eGFR, serum albumin, and urinary protein excretion to 4-OH-CPA exposure will enable a patient-directed approach to designing therapeutic regimens with cyclophosphamide.

There is considerable interest in the field of pharmacogenomics due to its contribution to drug pharmacokinetics and the promise of individualizing therapy to reduce variability in treatment responses. The pharmacogenetics of drug metabolism pathways involved in the breakdown of cyclophosphamide has been explored in patients with glomerulonephritis (Takada et al. 2004; Helsby et al. 2010; Joy et al. 2012). Takada et al. reported on the risk of premature ovarian failure and probability of reaching renal outcomes in lupus nephritis patients receiving cyclophosphamide (Takada et al. 2004). Patients exhibiting heterozygosity or homozygosity at the *CYP2C19 G681A* locus had reduced risk of premature ovarian failure. Patients who were homozygous for *CYP2B6 C1459T* or *CYP2C19 G681A* had increased probability of ESRD and doubling of serum creatinine. However, these homozygous variants have a low frequency in SLE populations, and the heterozygous variants did not associate with cyclophosphamide-induced lupus nephritis remissions (Winoto et al. 2011). These studies did not report on pharmacokinetics for either cyclophosphamide or 4-OH-CPA. The study by Joy et al. reported on the influence of the *CYP2B6 G516T* variant on the pharmacokinetics of cyclophosphamide. Variants had decreased maximal concentration (Cmax) in plasma after a dose and decreased elimination rate constant. Several trends were noted for the *ABCB1*/*MDR1* C3435T variant. The *ABCB1*/*MDR1 C3435T* variant exhibited a lower elimination rate constant and lower Cmax for cyclophosphamide. This study did not report on patient-related outcomes. Using both in vitro studies of liver samples from a biobank and plasma samples from 16 patients with SLE, Helsby et al. examined the role of the *CYP450* enzymes *CYP2B6* and *CYP2C19* in the bioactivation of cyclophosphamide (Helsby et al. 2010). They also examined the relationship of the 4-hydroxylation of cyclophosphamide to loss-of-function alleles in *CYP2B6* and *CYP2C19* in patients with lupus nephritis. Their studies suggest that one or more loss-of-function alleles in either *CYP2B6* or *CYP2C19* result in decreased bioactivation of cyclophosphamide. A few other studies have implicated variants of *CYP2B6* (Xie et al. 2006; Nakajima et al. 2007) and *CYP2C19* (Timm et al. 2005; Ekhart et al. 2008) in the decreased clearance of cyclophosphamide. The described studies support the approach of employing in vivo assessments of function of proteins and/or pharmacokinetics in evaluating the influence of genetic polymorphisms on any cyclophosphamide treatment-related outcomes.

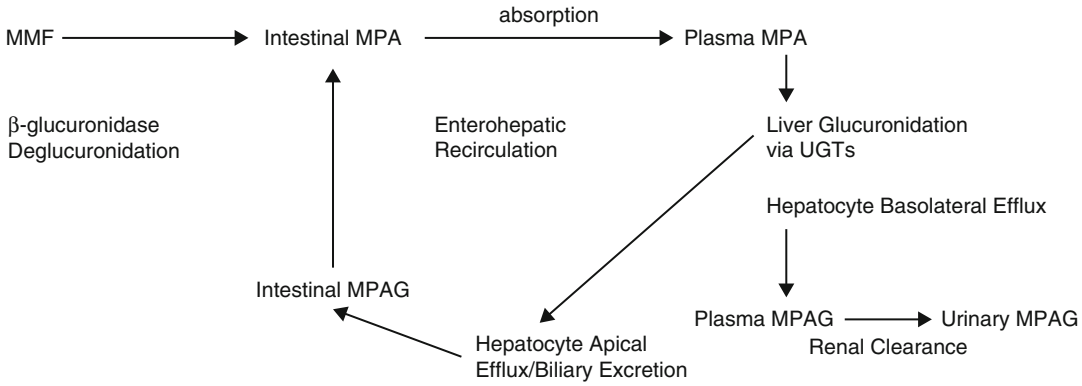
Mycophenolate

Mycophenolate, a drug commonly used in both induction and maintenance regimens of various forms of glomerulonephritis, is available as either the mofetil ester or the sodium salt. Mycophenolic acid (MPA) is the therapeutically



Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis, Fig. 3 Metabolic scheme for mycophenolate (Shipkova et al. 1999)

active moiety of both dosage forms. Mycophenolic acid is metabolized by a number of uridine diphosphate glucuronosyltransferase (UGT) enzymes to inactive glucuronide metabolites (MPAG) (Fig. 3). Over 90 % of the administered dose of mycophenolate is excreted in the urine, mostly as MPAG. The disposition of MPA is complicated, as MPAG undergoes active renal tubular secretion, as well as deglucuronidation in the intestines to result in a physiologic re-dosing of MPA known as enterohepatic recycling (Fig. 4). This later phenomenon is demonstrated by the appearance of two MPA peak concentrations, separated by several hours, after administration of the drug. The deconjugation process results in an estimated 40 % exposure (AUC) being attributable to enterohepatic circulation. Significant reductions in both eGFR and tubular secretion can lead to increased plasma levels of MPAG, which in turn



Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis, Fig. 4 Disposition pathway for mycophenolic acid (MPA)

can result in higher plasma concentrations of MPA through the availability of increased substrate for enterohepatic circulation. With regard to relevance of altered serum albumin concentrations and urinary protein excretion, about 97 % of MPA and 82–88 % of MPAG are bound to albumin (Joy et al. 2009c, d). At high concentrations of MPAG, such as might occur when there is impaired renal clearance, binding of MPA and MPAG to albumin is reduced. Studies have demonstrated reduced protein binding of MPA, with a bound fraction on the order of 94 % in patients with glomerulonephritis as compared with a mean of 97.5–99 % in renal transplant patients. The increased unbound concentration of MPA would contribute to enhanced pharmacologic and adverse effects.

Some studies evaluating the pharmacokinetics of MPA and/or its metabolites in patients with glomerulonephritis have been published (Mino et al. 2008a, b; Neumann et al. 2008; Joy et al. 2009c, d; Djabarouti et al. (2010), Lertdumrongluk et al. 2010; Sam and Joy 2010; Zahr et al. 2010; Mino et al. 2011a, b; Sagcal-Gironella et al. 2011). These studies suggest increased apparent oral clearance and corresponding decreased AUC for MPA in patients with glomerulonephritis versus renal transplant recipients. A study of patients with lupus nephritis looked at the influence of urinary protein excretion on the pharmacokinetics of MPA (Joy et al. 2009c). Patients with urinary protein excretion of $\geq$1 g/day had significantly increased apparent oral clearance and reduced AUC as compared with those with a urinary protein excretion $<$1 g/day. Higher urinary protein excretion was associated with reduced MPA trough concentrations in plasma. Multiple regression analyses were performed to determine which of several clinical factors in patients with glomerulonephritis (i.e., urine protein-to-creatinine ratio, creatinine clearance, serum albumin, age, race, gender, or steroid dose) had the greatest influence on the pharmacokinetics of MPA. Elevated creatinine clearance and reduced serum albumin significantly contributed to the apparent oral clearance of MPA. A glomerulonephritis population pharmacokinetics study that included patients with lupus nephritis and SVV demonstrated significant covariate effects of creatinine clearance and serum albumin on the pharmacokinetics of MPA and MPAG (Sam and Joy 2010). Importantly, this later study demonstrated that renal and nonrenal clearance parameters were affected by the creatinine clearance.

Lertdumrongluk et al. reported on the relationship between AUC and response to MPA in Asian patients with severe lupus nephritis (Lertdumrongluk et al. 2010). The patients in the study by Lertdumrongluk and colleagues were all Thai, and 85 % had nephropathy due to lupus. Treatment success, as defined by eGFR, inactive urine sediment, and reduction in urinary protein excretion, was associated with MPA exposures of $>$45 mg h/L. A study by

Zahr et al. reported on the relationship between MPA AUC and lupus disease activity as assessed by the SLE Disease Activity Index (SLEDAI) (Zahr et al. 2010). MPA exposure was negatively correlated with the results of the SLEDAI. The study reported that MPA plasma exposure levels of ≥35 mg h/L were associated with the lowest risk of active lupus. An additional study evaluated the relationship of MPA AUC to disease activity via the British Isles Lupus Assessment Groups Index (BILAG) in patients with childhood-onset lupus (Sagcal-Gironella et al. 2011). The patients in this study were of different races, and most of them had nephritis. The authors reported decreased (i.e., improved) BILAG scores on MPA therapy when AUC values were ≥30 mg h/L. A study of patients with SVV and lupus nephritis reported on the association between MPA trough plasma concentrations ≥3.5 mg/L and maintenance of disease remission (Neumann et al. 2008).

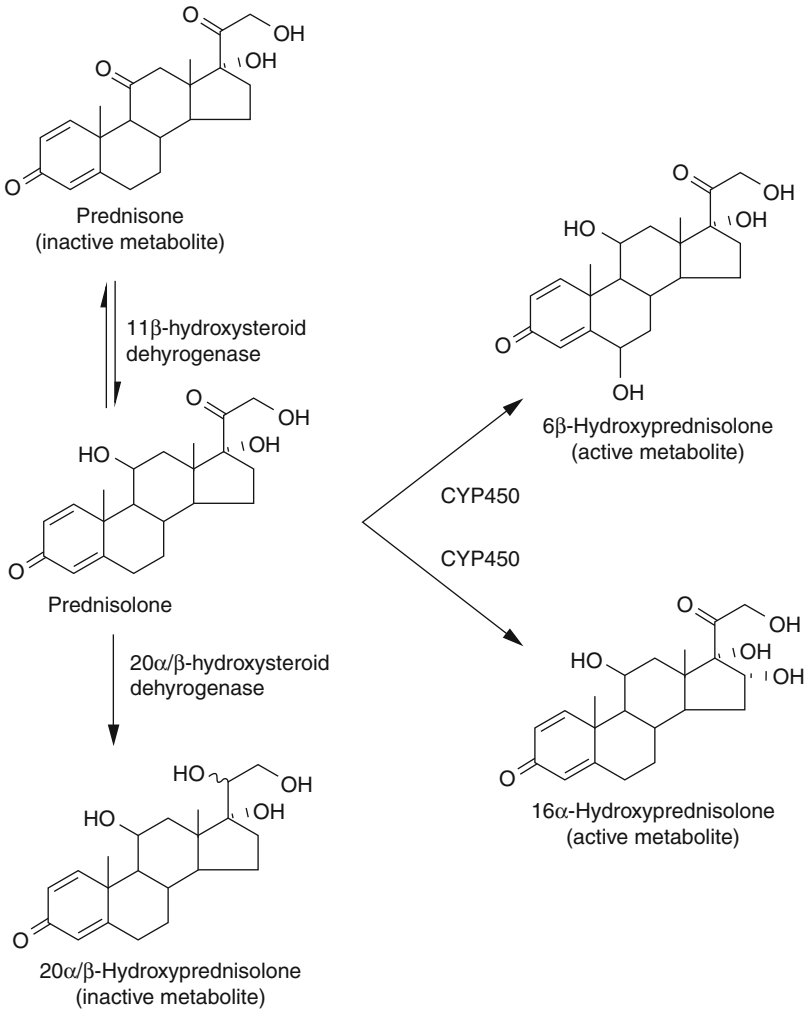
The influence of pharmacogenetics on MPA treatment responses in patients with glomerulonephritis has not yet been reported. However, it appears that polymorphisms in gene(s) encoding uridine diphosphate glucuronosyltransferases (UGTs) can influence the pharmacokinetics of MPA and MPAG (Joy et al. 2010a). Further research is needed to define the influence of pharmacogenomics on MPA efficacy and safety. The evolving data concerning the role of clinical covariates on MPA pharmacokinetics and MPA exposure targets to treatment responses provide evidence that personalized MPA therapy may be beneficial to patients with glomerulonephritis.

Glucocorticoids

The glucocorticoids are the most frequently used medications in the treatment of autoimmune diseases, including glomerulonephritis. Glucocorticoids have numerous mechanisms of action that contribute toward effectiveness in the treatment of autoimmune diseases, including suppression of inflammation, inhibition of T- and B-cell immunity, and alteration in the genetic regulation of proinflammatory cytokines. Despite their widespread use in glomerulonephritis, there is a dearth of information regarding the pharmacokinetics of glucocorticoids. Studies of prednisone and prednisolone, the most commonly used glucocorticoids, are complicated by the nature of their metabolism (Frey and Frey 1990) (Fig. 5). Prednisone is a prodrug that is orally administered. Prednisolone is not stable as an oral formulation and hence is available as a parenteral product employing various salts. Prednisolone is the active form of the drug. The 11beta-hydroxysteroid dehydrogenase is responsible for converting the prodrug prednisone to prednisolone. A subsequent dehydrogenase step results in the formation of an inactive metabolite. However, metabolism via cytochrome P450s results in the formation of additional active metabolites. Notably, both prednisone and prednisolone undergo reversible metabolism resulting in formation clearance of prednisolone from prednisone and vice versa. This section will review basic glucocorticoid pharmacokinetics, as well as the available data pertaining to nephrotic syndrome and glomerulonephritis.

A study by Garg and Jusko attempted to evaluate the pharmacokinetics of prednisone and prednisolone in healthy male subjects (Garg and Jusko, 1994). In this double-blind, randomized, two-way crossover study, subjects were treated with oral prednisone (0.8 mg/kg) and parenteral prednisolone sodium phosphate (equivalent to 0.66 mg/kg of the free base). Regardless of the product evaluated, prednisolone concentrations exceeded that of prednisone at each time point evaluated. Poor oral absorption of prednisone accounted for the lower bioavailability of prednisolone derived from prednisone. The formation clearance of prednisolone from prednisone was 10-times greater than the formation clearance of prednisone from prednisolone.

These findings conflict somewhat with those of an earlier study by Rocci et al. (Rocci et al. 1982). The pharmacokinetics of prednisone and prednisolone were evaluated in six children with nephrotic syndrome. The exposure of prednisolone was significantly higher after oral prednisone than after intravenous prednisolone, suggesting that the oral absorption of prednisone



Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis, Fig. 5 Metabolic scheme of prednisone and prednisolone (Foye's Principles of Medicinal Chemistry Fig. 33.12; Miller et al. (2008))

and its subsequent conversion are not altered in children with nephrotic syndrome. They also reported increased apparent clearance of prednisolone as compared to previous reports in children with asthma. Renal clearance of both prednisone and prednisolone were found to be normal, suggesting that loss of protein-bound drug in the urine was not problematic. However, since steroids are highly lipophilic and bind to lipoproteins as well as to transcortin and albumin, assessing the influence of proteinuria on renal clearance may be difficult to fully characterize.

Several published studies in nephrotic patients sought to determine if glucocorticoid pharmacokinetics correlated with clinical response or side effects. A study by Rostin et al. demonstrated that the AUC and peak plasma concentrations of prednisolone were highly correlated with serum albumin concentrations, as might be expected for a drug that is bound to albumin (Rostin et al. 1990). These results supported a previous study reporting lower serum prednisolone concentrations in patients with nephrotic syndrome (Frey and Frey 1982). However, correlations of pharmacokinetic parameters to clinical effectiveness could not be identified by Rostin and colleagues.

An earlier study sought to identify a relationship between pharmacokinetic factors and steroid responsiveness in children with nephrotic syndrome (Gatti et al. 1984). Children were assessed during the active phase of disease

and during remission. The authors noted that total serum prednisolone levels during the active phase of disease were significantly lower than during remission. The free fraction of prednisolone was found to correlate negatively with serum albumin levels and was increased during the active phase of disease. However, free prednisolone levels were not significantly different during the active phase and remission, suggesting that the concentration of prednisolone available at pharmacologic sites of action is not different according to disease activity. A recent study reported the pharmacokinetics of prednisolone in eight patients with childhood-onset lupus without changes in GFR (Sagcal-Gironella et al. 2011). The authors sought to correlate drug exposure with disease activity. The results demonstrated that high AUCs were associated with low disease activity as measured by the BILAG. While the limited studies (with small patient numbers) of prednisolone pharmacokinetics in nephrotic syndrome and lupus suggest some alterations and correlation with disease activity, there are multiple patient confounders that have not been appropriately assessed. There are no publications describing the role of pharmacogenomics in drug disposition genes on prednisone and prednisolone responses in glomerulonephritis. This is relevant since numerous dehydrogenases, CYPs, and drug transporters are important for the disposition of these agents. Patients prescribed glucocorticoids for the treatment of glomerulonephritis are often on other medications with documented drug interactions that may alter response or safety. Glucocorticoids are known to induce the metabolism of numerous medications, resulting in altered therapeutic efficacy and safety of the victim drug.

Monoclonal Antibodies

Monoclonal antibodies are increasingly being investigated to treat diseases associated with glomerulonephritis. Rituximab is a monoclonal antibody that received US Food and Drug Administration approval on April 29, 2011, for treatment of GPA and microscopic polyangiitis (MPA), two forms of anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis. A second monoclonal antibody, adalimumab, has been studied in patients with FSGS (Joy et al. 2010b). Infliximab has been used as a third-line agent to treat refractory lupus nephritis.

All subclasses of human IgG have a molecular weight of 145 kD, approximately twice the molecular weight of albumin (67 kD). Based on the molecular weight, therapeutic antibodies would not be expected to be excreted in the urine. However, proteinuric kidney diseases, such as glomerulonephritis, can result in urinary excretion of large amounts of proteins. While albumin is the predominant constituent of the proteins found in the urine, there is evidence of loss of other macromolecular proteins, especially in the setting of nephrotic-range proteinuria ($\geq$3 g protein/day). A study of pediatric patients with nephrotic syndrome due to minimal change disease (MCD), focal glomerulosclerosis (FGS), or membranoproliferative glomerulonephritis (MPGN) found that many patients with MCD and FGS had significantly decreased serum levels of immunoglobulins (Ellis and Buffone 1981). For patients with MCD, there were only minimal urinary immunoglobulins, suggesting either decreased production or increased systemic clearance via nonrenal routes. Conversely, patients with FGS had high urinary IgG excretion relative to serum levels. These data provided the first suggestion that nephrotic patients have increased systemic clearance of immunoglobulins through renal and nonrenal routes of elimination.

Rituximab is an intravenously administered, CD20-specific chimeric murine/human monoclonal antibody that selectively targets B cells at all phases of maturation, with the exception of the earliest and latest phases, including plasma cells. CD20 is a B-cell differentiation marker not found on most other cells, and it is not found in the circulation. Rituximab represents a promising rational treatment for many forms of autoimmune disease, since it selectively targets B cells. In the case of ANCA-associated glomerulonephritis, rituximab targets the B cells that have been clearly demonstrated to play a pathogenic role in the development and recurrence of disease.

Clinical studies have reported that rituximab is non-inferior to cyclophosphamide for initial therapy or recurrence of either GPA or MPA in both short-term (Jones et al. 2010; Specks and Stone 2010) and long-term follow-up (Jones et al. 2011; Specks et al. 2011). The antibody is composed of two heavy chains of 451 amino acids and two light chains of 213 amino acids, with a resultant molecular weight of 145 kD. This size is consistent with normal human IgG molecules, which generally have a weight of 146 kD (IgG1, 2, and 4), or in the case of IgG3 of 170 kD. Pharmacokinetic data for rituximab in patients with kidney disease of any etiology is generally lacking. There are few case reports suggesting that target levels of the drug can be achieved both in patients with chronic kidney disease, as well as end-stage renal disease, without adverse effect (Castro et al. 2001; Jillella et al. 2002). However, significant data supporting these observations are unavailable. Multiple studies have shown rituximab to be highly effective in reducing proteinuria secondary to several disease entities, and there is evidence that rituximab can reverse glomerular pathology in patients with nephrotic-range proteinuria. Studies to determine the influence of proteinuria on renal and nonrenal clearance and other pharmacokinetics parameters, as well as to determine targets for drug exposure and therapeutic dosing strategies, are needed to maximize beneficial effects and minimize potential adverse effects.

Adalimumab is a subcutaneously administered, fully humanized recombinant monoclonal antibody of the IgG1 subclass, with high affinity for tumor necrosis factor alpha (TNFα). It comprises 133 amino acids and has a molecular weight of approximately 148 kD, similar to that of rituximab. The recently reported FONT Study (Novel Therapies for Resistant FSGS) was designed to investigate the pharmacokinetic characteristics, tolerability, and safety of pharmaceutical agents with potential for antifibrotic activity (Joy et al. 2010b). Adalimumab was one of the drugs studied in the setting of patients with resistant primary FSGS. The FONT investigators found a twofold higher apparent clearance and twofold lower AUC in the proteinuric patients with FSGS as compared to previous reports in patients with rheumatoid arthritis. A shorter time to maximum plasma concentration (Tmax) and lower maximum plasma concentration (Cmax) were also reported as compared to patients with rheumatoid arthritis. Preliminary unpublished data suggest increased renal and nonrenal clearance of adalimumab in FSGS. The data also suggested that patients who were able to achieve a predefined targeted trough plasma concentration (Ctrough) demonstrated a better therapeutic response as defined by decreased urinary protein excretion and increased eGFR. The data demonstrating the pharmacokinetics of adalimumab in patients with proteinuria suggest the need for thorough clinical pharmacology studies when therapeutic proteins are being considered for use in proteinuric kidney diseases in order to appropriately define and meet targeted exposure criteria to maximize treatment responses.

The drugs discussed here represent only a few of the medications now available as part of the armamentarium for treatment of SVV, SLE, and other forms of glomerular disease. Discussion of these particular drugs highlights some of the issues that remain in understanding how best to utilize them. Of particular importance is a better understanding of the clinical pharmacology (pharmacokinetics) of these drugs, particularly in the setting of the diseases that they are used to treat. By further elucidating the specifics of altered drug-related parameters (clearance, volume of distribution, AUC), clinical factors (proteinuria, GFR, serum albumin) and level of inflammation, and the role of genetic factors and drug interactions, drugs investigated for use in glomerulonephritis may show even greater promise for ameliorating or even curing a number of diseases that continue to cause significant morbidity and mortality.

Cross-References

- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Systemic Lupus Erythematosus, Clinical Trials
- ▶ Vasculitis and the Kidney

References

Belfayol-Pisanté L, Guillevin L, Tod M, Fauvelle F. Pharmacokinetics of cyclophosphamide (CP) and 4-OH-CP/aldophosphamide in systemic vasculitis. Fundam Clin Pharmacol. 2000;14(4):415–21.

Castro M, Van Auken J, Sponzo R. Successful use of anti-CD20 monoclonal antibody, Rituximab, in patients with non-Hodgkin's lymphoma (NHL) and renal insufficiency [Abstract]. Proc Am Soc Clin Oncol. 2001;20:233b.

Chadban SJ, Briganti EM, Kerr PG, Dunstan DW, Welborn TA, Zimmet PZ, Atkins RC. Prevalence of kidney damage in Australian adults: the AusDiab kidney study. J Am Soc Nephrol. 2003;14:S131.

Choi HS, Sung KC, Lee KB. The prevalence and risk factors of microalbuminuria in normoglycemic, normotensive adults. Clin Nephrol. 2006;65:256.

de Miranda Silva C, Fernandes BJ, Donadi EA, Silva LM, Coelho EB, Dantas M, Marques MP, Lanchote VL. Influence of glomerular filtration rate on the pharmacokinetics of cyclophosphamide enantiomers in patients with lupus nephritis. J Clin Pharmacol. 2009;49(8):965–72.

Djabarouti S, Breilh D, Duffau P, Lazaro E, Greib C, Caubet O, Saux MC, Pellegrin JL, Viallard JF. Steady-state mycophenolate mofetil pharmacokinetic parameters enable prediction of systemic lupus erythematosus clinical flares: an observational cohort study. Arthritis Res Ther. 2010;12(6):R217.

Dooley MA, Hogan S, Jennette C, Falk R. Cyclophosphamide therapy for lupus nephritis: poor renal survival in black Americans. Glomerular Disease Collaborative Network. Kidney Int. 1997;51:1188–95.

Ekhart C, Doodeman VD, Rodenhuis S, Smits PH, Beijnen JH, Huitema AD. Influence of polymorphisms of drug metabolizing enzymes (CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, GSTA1, GSTP1, ALDH1A1 and ALDH3A1) on the pharmacokinetics of cyclophosphamide and 4-hydroxycyclophosphamide. Pharmacogenet Genomics. 2008;18:515–23.

Ellis D, Buffone GJ. Protein clearances and selectivity determinations in childhood nephrosis: a reappraisal. Clin Chem. 1981;27(8):1397–400.

Fahey JL, Leonard E, Churg J, Godman G. Wegener's granulomatosis. Am J Med. 1954;17(2):168–79.

Frey BM, Frey FJ. Clinical pharmacokinetics of prednisone and prednisolone. Clin Pharmacokinet. 1990;19(2):126–46.

Frey FJ, Frey BM. Altered prednisolone kinetics in patients with the nephrotic syndrome. Nephron. 1982;32(1):45–8.

Garg V, Jusko WJ. Bioavailability and reversible metabolism of prednisone and prednisolone in man. Biopharm Drug Dispos. 1994;15(2):163–72.

Gatti G, Perucca E, Frigo GM, Notarangelo LD, Barberis L, Martini A. Pharmacokinetics of prednisone and its metabolite prednisolone in children with nephrotic syndrome during the active phase and in remission. Br J Clin Pharmacol. 1984;17(4):423–31.

Haubitz M, Bohnenstengel F, Brunkhorst R, Schwab M, Hofmann U, Busse D. Cyclophosphamide pharmacokinetics and dose requirements in patients with renal insufficiency. Kidney Int. 2002;61(4):1495–501.

Hebert LA, Parikh SV. Differential diagonis and evaluation of glomerular disease. UpToDate 2012.

Helsby NA, Hui CY, Goldthorpe MA, Coller JK, Soh MC, Gow PJ, De Zoysa JZ, Tingle MD. The combined impact of CYP2C19 and CYP2B6 pharmacogenetics on cyclophosphamide bioactivation. Br J Clin Pharmacol. 2010;70(6):844–53.

Hunder GG. Classification of and approach to the vasculitides in adults. UpToDate. 2013.

Jafar TH, Schmid CH, Levey AS. Serum creatinine as marker of kidney function in South Asians: a study of reduced GFR in adults in Pakistan. J Am Soc Nephrol. 2005;16:1413.

Jillella AP, Dainer PM, Kallab AM, Ustun C. Treatment of a patient with end-stage renal disease with Rituximab: pharmacokinetic evaluation suggests Rituximab is not eliminated by hemodialysis. Am J Hematol. 2002;71(3):219–22.

Jones RB, Tervaert JW, Hauser T, Luqmani R, Morgan MD, Peh CA, Savage CO, Segelmark M, Tesar V, van Paassen P, Walsh D, Walsh M, Westman K, Jayne DR, European Vasculitis Study Group. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. N Engl J Med. 2010;363(3):211.

Jones RB, Walsh M, Jayne DRW, European Vasculitis Study Group. Two-year follow-up results from a randomized trial of RTX versus CyP for ANCA-associated renal vasculitis: RITUXVAS. Clin Exp Immunol. 2011;164:57.

Joy MS. Impact of glomerular kidney diseases on the clearance of drugs. J Clin Pharmacol. 2011;52(Supp 1):23S–34.

Joy MS, Dornbrook-Lavender K, Blaisdell J, Hilliard T, Boyette T, Hu Y, Hogan SL, Candiani C, Falk RJ, Goldstein JA. CYP2C9 genotype and pharmacodynamic responses to losartan in patients with primary and secondary kidney diseases. Eur J Clin Pharmacol. 2009a;65(9):947–53.

Joy MS, Gipson DS, Dike M, Powell L, Thompson A, Vento S, Eddy A, Fogo AB, Kopp JB, Cattran D, Trachtman H. Phase I trial of rosiglitazone in FSGS: I. Report of the FONT Study Group. Clin J Am Soc Nephrol. 2009b;4(1):39–47. Epub 2008 Dec 10.

Joy MS, Hilliard T, Hu Y, Hogan SL, Dooley MA, Falk RJ, Smith PC. Pharmacokinetics of mycophenolic acid in patients with lupus nephritis. Pharmacotherapy. 2009c;29:7–16.

Joy MS, Hilliard T, Hu Y, Hogan SL, Wang J, Falk RJ, Smith PC. Influence of clinical and demographic variables on mycophenolic acid pharmacokinetics in antineutrophil cytoplasmic antibody-associated vasculitis. Ann Pharmacother. 2009d;43:1020–7.

Joy MS, Boyette T, Hu Y, Wang J, La M, Hogan SL, Stewart PW, Falk RJ, Dooley MA, Smith PC. Effects of uridine diphosphate glucuronosyltransferase 2B7 and 1A7 pharmacogenomics and patient clinical parameters on steady-state mycophenolic acid pharmacokinetics in glomerulonephritis. Eur J Clin Pharmacol. 2010a;66(11):1119–30.

Joy MS, Gipson DS, Powell L, MacHardy J, Jennette JC, Vento S, Pan C, Savin V, Eddy A, Fogo AB, Kopp JB, Cattran D, Trachtman H. Phase 1 trial of adalimumab in Focal Segmental Glomerulosclerosis (FSGS): II. Report of the FONT (Novel Therapies for Resistant FSGS) study group. Am J Kidney Dis. 2010b;55(1):50–60. Epub 2009 Nov 22.

Joy MS, La M, Wang J, Bridges AS, Hu Y, Hogan SL, Frye RF, Blaisdell J, Glodstein JA, Dooley MA, Brouwer KL, Falk RJ. Cyclophosphamide and 4-hydroxycyclophosphamide pharmacokinetics in patients with glomerulonephritis secondary to lupus and small vessel vasculitis. Br J Clin Pharmacol. 2012;74(3):445–55.

Lertdumrongluk P, Somparn P, Kittanamongkolchai W, Traitanon O, Vadcharavivad S, Avihingsanon Y. Pharmacokinetics of mycophenolic acid in severe lupus nephritis. Kidney Int. 2010;78(4):389–95.

Miller DD, Brueggemeier RW, Dalton JT. Adrenocorticoids, Ch. 33, p. 891. In: Lemke TL, Williams DA, Roche VF, Zito SW, editors. Foye's principles of medicinal chemistry. 6th ed. Baltimore: Lippincott Williams & Wilkins; 2008.

Mino Y, Naito T, Matsushita T, Otsuka A, Ushiyama T, Ozono S, Hishida A, Kagawa Y, Kawakami J. Comparison of pharmacokinetics of mycophenolic acid and its glucuronide between patients with lupus nephritis and with kidney transplantation. Ther Drug Monit. 2008a;30(6):656–61.

Mino Y, Naito T, Matsushita T, Kagawa Y, Kawakami J. Simultaneous determination of mycophenolic acid and its glucuronides in human plasma using isocratic ion pair high-performance liquid chromatography. J Pharm Biomed Anal. 2008b;46(3):603–8.

Mino Y, Naito T, Shimoyama K, Ogawa N, Kawakami J. Pharmacokinetic variability of mycophenolic acid and its glucuronide in systemic lupus erythematosus patients in remission maintenance phase. Biol Pharm Bull. 2011a;34(5):755–9.

Mino Y, Naito T, Shimoyama K, Ogawa N, Kawakami J. Effective plasma concentrations of mycophenolic acid and its glucuronide in systemic lupus erythematosus patients in the remission-maintenance phase. J Clin Pharm Ther. 2011; [Epub ahead of print].

Nakajima M, Komagat S, Fujiki Y, Kanada Y, Ebi H, Itoh K, Mukai H, Yokoi T, Minami H. Genetic polymorphisms of CYP 2B6 affect the pharmacokinetics/pharmacodynamics of cyclophosphamide in Japanese cancer patients. Pharmacogenet Genomics. 2007;17:431–45.

Neumann I, Fuhrmann H, Kanzler M, Fang IF, Jaeger A, Graf H, Bayer P, Kovarik J. Pharmacokinetics of enteric-coated mycophenolate sodium: comparative study in patients with autoimmune disease and renal allograft. Expert Opin Pharmacother. 2008;9(6):879–86.

Nolin TD, Naud J, Leblond FA, Pichette V. Emerging evidence of the impact of kidney disease on drug metabolism and transport. Clin Pharm Ther. 2008;83(6):898–903.

Rocci Jr ML, Assael BM, Appiani AC, Edefonti A, Jusko WJ. Effect on nephrotic syndrome on absorption and disposition of prednisolone in children. Int J Pediatr Nephrol. 1982;3(3):159–66.

Rostin M, Barthe P, Houin G, Alvinerie M, Bouissou F. Pharmacokinetics of prednisolone in children with the nephrotic syndrome. Pediatr Nephrol. 1990;4(5):470–3.

Sagcal-Gironella AC, Fukuda T, Wiers K, Cox S, Nelson S, Dina B, Sherwin CM, Klein-Gitelman MS, Vinks AA, Brunner HI. Pharmacokinetics and pharmacodynamics of mycophenolic acid and their relation to response to therapy of childhood-onset systemic lupus erythematosus. Semin Arthritis Rheum. 2011;40(4):307–13.

Sam WJ, Joy MS. Population pharmacokinetics of mycophenolic acid and metabolites in patients with glomerulonephritis. Ther Drug Monit. 2010;32:594–605.

Shipkova M, Armstrong VW, Wieland E, Niedmann PD, Schutz E, Brenner-Weiss G, Voihsel M, Braun F, Oellerich M. Identification of glucoside and carboxyl-linked glucuronide conjugates of mycophenolic acid in plasma of transplant recipients treated with mycophenolate mofetil. Br J Pharmacol. 1999;126(5):1075–82.

Specks U, Stone JH, RAVE-ITN Research Group. Long-term efficacy and safety results of the RAVE trial. Clin Exp Immunol. 2011;164:65.

Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, Kallenberg CG, St Clair EW, Turkiewicz A, Tchao NK, Webber L, Ding L, Sejismundo LP, Mieras K, Weitzenkamp D, Ikle D, Seyfert-Margolis V, Mueller M, Brunetta P, Allen NB, Fervenza FC, Geetha D, Keogh KA, Kissin EY, Monach PA, Peikert T, Stegeman C, Ytterberg SR, Specks U, RAVE-ITN Research Group. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med. 2010;363(3):221.

Takada K, Arefayene M, Desta Z, Yarboro CH, Boumpas DT, Balow JE, Flockhart DA, Illei GG. Cytochrome P450 pharmacogenetics as a predictor of toxicity and clinical response to pulse cyclophosphamide in lupus nephritis. Arthritis Rheum. 2004;50(7):2202–10.

Timm R, Kaiser R, Lotsch J, Heider U, Sezer O, Weisz K, Montemurro M, Roots I, Cascorbi I. Association of cyclophosphamide pharmacokinetics to polymorphic cytochrome P450 2C19. Pharmacogenomics J. 2005;5:365–73.

U.S. Department of Health and Human Services: National Kidney & Urologic Diseases Information Clearinghouse (NKUDIC). http://kidney.niddk.nih.gov/kudiseases/pubs/kustats (2011). Accessed 15 Sept 2011.

Winoto J, Song H, Hines C, Nagaraja H, Rovin BH. Cytochrome P450 polymorphisms and the response of lupus nephritis to cyclophosphamide therapy. Clin Nephrol. 2011;75(5):451–7.

Xie H, Griskevicius L, Ståhle L, Hassan Z, Yasar U, Rane A, Broberg U, Kimby E, Hassan M. Pharmacogenetics of cyclophosphamide in patients with hematological malignancies. Eur J Pharm Sci. 2006;27:54–61.

Zahr N, Arnaud L, Marquet P, Haroche J, Costedoat-Chalumeau N, Hulot JS, Funck-Brentano C, Piette JC, Amoura Z. Mycophenolic acid area under the curve correlates with disease activity in lupus patients treated with mycophenolate mofetil. Arthritis Rheum. 2010;62(7):2047–54.

Translational Aspects of Membranous Nephropathy

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Synonyms

Chronic kidney disease; Class V lupus nephritis; Idiopathic nephrotic syndrome; Membranous glomerulonephritis; Nephrotic syndrome; Proteinuria

Definition

Membranous nephropathy (MN) refers to a pathologic pattern of injury to the filtering units in the kidney, the renal glomeruli, characterized by minimal changes (early) or glomerular basement membrane (GBM) thickening (later) by light microscopy; diffuse, finely granular deposits of IgG and complement C3 exclusively along the outer surface of the capillary wall by immunofluorescence microscopy (IF); and corresponding diffuse electron-dense deposits in an exclusively subepithelial distribution by electron microscopy (EM) (Couser and Cattran 2010; Fervenza et al. 2008). MN is a pathologic diagnosis made when a renal biopsy is performed to evaluate the cause of an unexplained increase in excretion of protein in the urine. In such patients MN is the underlying disease in 10 % of children, 33 % of adults, and 40 % of adults over 60. About 80 % of cases of MN are without obvious etiology (idiopathic, IMN) (Couser and Cattran 2010a; Fervenza et al. 2008). Most of these are autoimmune caused by glomerular deposition of an IgG_4 autoantibody to M-type phospholipase A2 receptor (PLA2R), a membrane glycoprotein expressed in the kidney only by glomerular podocytes that line the outer surface of the glomerular capillary wall (Beck et al. 2009).

In the remaining 20 % of cases, the lesion of MN develops in a setting of some systemic disease (secondary MN) (Table 1). In secondary MN, the antigens involved are non-glomerular but may also be self-antigens as seen, for example, in lupus (class V lupus nephritis) (Couser and Cattran 2010a; Fervenza et al. 2008).

Epidemiology

In the USA, the incidence of end-stage renal disease (ESRD) due to MN is about 460 patients/year, which represents 0.4 % of the total end-stage renal disease (ESRD) population (Couser and Cattran 2010; Fervenza et al. 2008). Since only about 20 % of all patients with MN now progress to ESRD, the real incidence of MN is likely about 2,300 patients per year in the USA or about eight patients/million population/year (Couser and Cattran 2010a; Fervenza et al. 2008).

The risk for MN is increased threefold in Caucasian patients with HLA-DR3, and associations with HLA-B8 and HLA-B18 have also been reported. The risk of progressive loss of renal function in Caucasians is increased with HLA-DR5 and HLA-DR3. In Japan, MN is associated with HLA-DR2. Some Caucasian patients have a deletion of C4 with the HLA-B8–DR3 haplotype (Couser and Cattran 2010). Familial MN is rare and is usually seen in children. Genome-wide association studies have also identified a link between IMN and the gene for PLA2R. The presence of both HLA and PLA2R risk alleles increases the odds of developing IMN almost 80 fold (Stanescu et al. 2011).

Translational Aspects of Membranous Nephropathy, Table 1 Causes of secondary membranous nephropathy (Adapted with permission from Couser and Cattran (2010). All permissions were obtained through ScienceDirect at www.sciencedirect.com)

Groups	Common	Uncommon
Immune diseases	Systemic lupus erythematosus, diabetes mellitus	Rheumatoid arthritis, Hashimoto's disease, Graves' disease, mixed connective tissue disease, Sjögren's syndrome, primary biliary cirrhosis, bullous pemphigoid, small bowel enteropathy syndrome, dermatitis herpetiformis, ankylosing spondylitis, graft-versus-host disease, Guillain–Barré syndrome, bone marrow and stem cell transplantation, anti-GBM and ANCA-positive crescentic glomerulonephritis
Infectious or parasitic diseases	Hepatitis B	Hepatitis C, syphilis, filariasis, hydatid disease, schistosomiasis, malaria, leprosy
Drugs and toxins	Gold, penicillamine, nonsteroidal anti-inflammatory drugs	Mercury, captopril, formaldehyde, hydrocarbons, bucillamine agents
Miscellaneous	Tumors, renal transplantation	Sarcoidosis, sickle cell disease, Kimura disease, angiofollicular lymph node hyperplasia

The list excludes conditions where only a single case has been reported or where the lesions were atypical of membranous nephropathy

Clinical Features

Common clinical features in IMN are summarized in Table 2. In adults, about 70 % of patients with IMN present with nephrotic syndrome, a clinical diagnosis made when the measured urine protein excretion exceeds 3.5 g/day or the ratio of protein to creatinine in a random urine sample exceeds 3.0 (Couser and Cattran 2010a; Fervenza et al. 2008; Hladunewich et al. 2009). Renal function is normal at presentation in over 90 %. Many such patients exhibit other disease manifestations including reduced serum albumin levels, elevated lipid levels, and peripheral edema, collectively referred to as nephrotic syndrome. About 25 % present with elevated but lower levels of protein excretion. Sixty percent of these patients progress to nephrotic syndrome, usually in the first year after diagnosis, and 10 % of all IMN patients never become nephrotic (Hladunewich et al. 2009). Spontaneous remissions with disappearance of the proteinuria without therapy occur in about 32 % in an average of 14 months (Couser and Cattran 2010a; Fervenza et al. 2008; Hladunewich et al. 2009).

About 80 % of cases of MN represent "idiopathic" primary renal diseases, and most are now recognized to be consequent to an autoimmune mechanism caused by development of an autoantibody to a podocyte membrane protein (phospholipase A2 receptor (PLA2R)) (Couser and Cattran 2010a; Beck et al. 2009; Fervenza et al. 2008) (see below). About 20 % of patients have secondary MN which may also involve nonrenal self-antigens (lupus) or exogenous non-self-antigens as is presumed to occur in MN associated with hepatitis B and C virus

Translational Aspects of Membranous Nephropathy, Table 2 Clinical features of idiopathic membranous nephropathy (Adapted with permission from Couser and Cattran (2010). All permissions were obtained through ScienceDirect at www.sciencedirect.com)

Rare in children – < 5 % of total cases of nephrotic syndrome
Common in adults – 15–50 % of total cases of nephrotic syndrome, depending on age. Increasing frequency after age 40 years, commonest cause in adults >60
Males > females in all adult groups
Caucasians > Asians > African–Americans > Hispanics
Nephrotic syndrome in 60–70 %
Normal or mildly elevated BP at presentation
"Benign" urinary sediment
Nonselective proteinuria
Tendency to thromboembolic disease
Secondary causes: infection, drugs, neoplasia, systemic lupus erythematosus

infection, various chronic parasitic infections, drug exposures, malignancies, and (in infants) allergy to cow's milk (Couser and Cattran 2010a; Fervenza et al. 2008; Hladunewich et al. 2009). The commonest causes of secondary MN are listed in Table 1. An increased incidence of MN has been reported in association with other autoimmune diseases, particularly type I diabetes, anti-GBM disease, ANCA-associated vasculitis, and lupus. Lupus MN is anti-PLA2R negative, but the nature of the autoantibody in these secondary autoimmune forms of the disease is otherwise unknown.

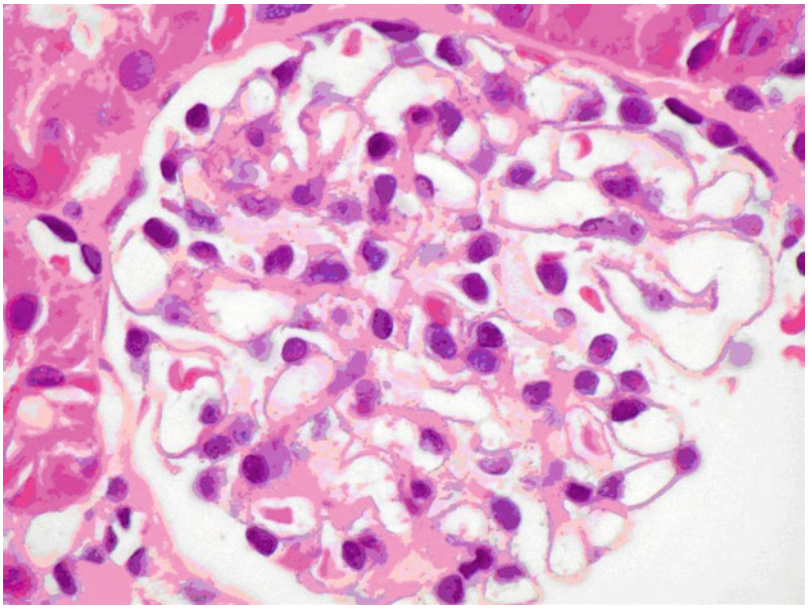
The clinical consequences of MN can be considered as both short and long term. In the short term, they reflect the result of an increased loss of albumin and other serum proteins in the urine that can lead to hypoalbuminemia with salt and water retention resulting in edema and even anasarca, a high risk for development of thrombotic and thromboembolic events (10–40 %) due to loss of coagulation inhibitors in the urine and increased risk of infection due primarily to urinary loss of immunoglobulins (Couser and Cattran 2010a; Fervenza et al. 2008; Hladunewich et al. 2009) (Table 2). However, the most feared long-term consequence of MN is progressive loss of renal function leading to ESRD. This course is followed by about 50 % of patients with 35 % developing ESRD within 10 years, 41 % at 15 years, and another 10 % after that (Couser and Cattran 2010a; Fervenza et al. 2008; Hladunewich et al. 2009). The risk of progression is directly related to the level of urine protein excretion over time. Progression occurs in <5 % of patients who never develop nephrotic-range proteinuria, <10 % of those who maintain protein excretions below 4 g/day, 50 % of those with persistent levels of 4–8 g/day, and 70–80 % of those who continue to excrete 8–10 or more grams/day (Couser and Cattran 2010a; Fervenza et al. 2008; Hladunewich et al. 2009). Other risk factors for progression include male sex, elevated blood pressure, and likely persistently elevated levels of anti-PLA2R antibodies.

In secondary MN, the course will vary depending on the etiology and success in treating the underlying primary disease (Table 1). For example, drug-induced disease usually resolves spontaneously with discontinuation of the drug, viral infection-related MN can resolve with successful antiviral therapy, cancer-associated MN has been reported to resolve with removal of the tumor, and lupus-associated MN follows a chronic course that usually parallels the course of the systemic lupus (Couser and Cattran 2010; Fervenza et al. 2008; Hladunewich et al. 2009).

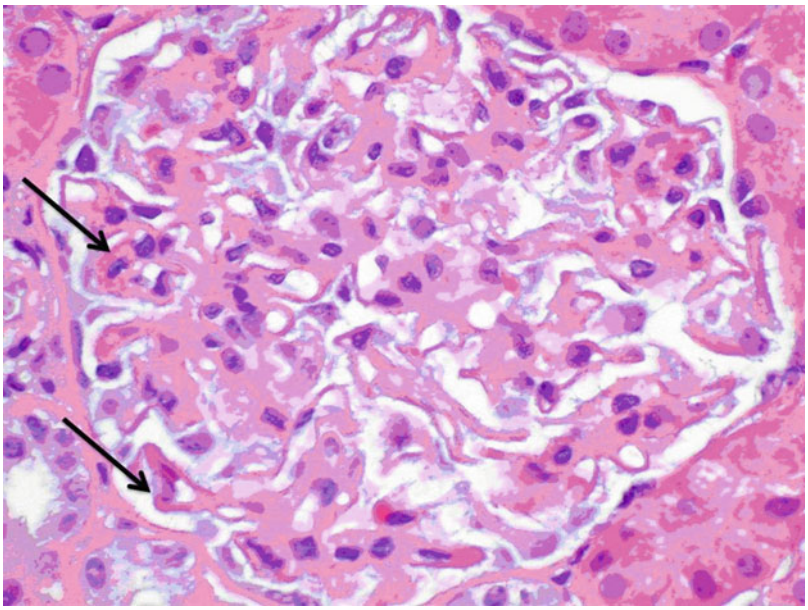
Pathology

As mentioned above, MN is a pathology diagnosis made by study of a diagnostic renal biopsy. The diagnostic pathologic changes are seen only in glomeruli and are uniform involving all glomeruli to the same degree (Couser and Cattran 2010a; Fervenza et al. 2008). By light microscopy, glomeruli may appear entirely normal in early disease (Fig. 1). With progression changes of basement membrane, thickening (hence the term membranous) (Fig. 2) with formation of subepithelial "spikes" of basement membrane on the outer surface of the capillary wall become apparent, particularly when a stain that marks extracellular matrix such as silver methenamine is employed (Fig. 3).

IF reveals diffuse, uniform, (Kanigicherla et al. 2013) finely granular deposits of IgG and C3 along the outer surface of the capillary wall in all glomeruli (Fig. 4) (Couser and Cattran 2010; Fervenza et al. 2008). The predominant IgG subtype in IMN is IgG_4 usually reflecting the deposition of IgG_4 anti-PLA2R antibody, although lesser deposits of IgG_1 and IgG_3 may be seen (Beck et al. 2009). The presence of primarily IgG_1 or IgG_3, IgM or IgA, suggests a secondary cause, particularly lupus, in which a "full house" IF pattern is typical (Couser and Cattran 2010a; Fervenza et al. 2008). Complement deposits include C3, C4, and C5b-9 but not C1q consistent with complement activation by either the MBL or alternate complement pathways (see below) (Couser 2012; Cybulsky 2011). PLA2R is also a constituent of the subepithelial deposits in most cases studied (Beck et al. 2009). A variety of nonrenal antigens



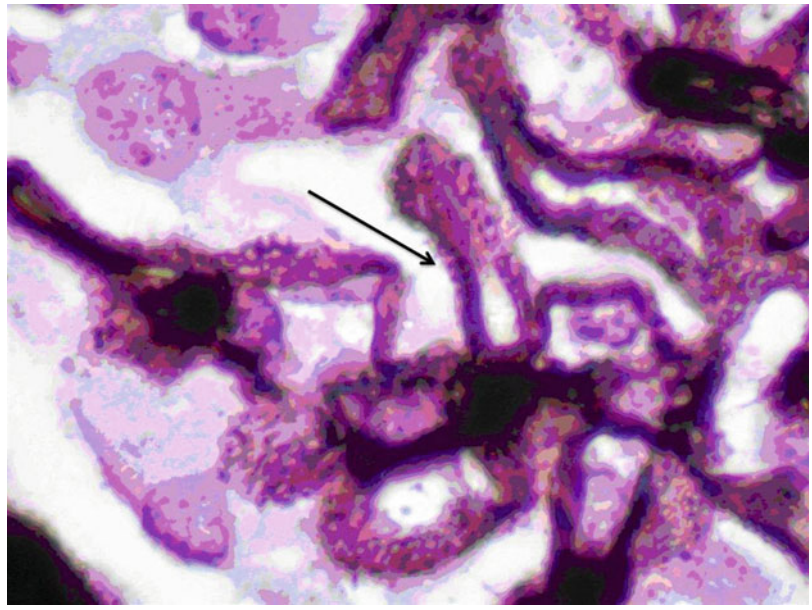
Translational Aspects of Membranous Nephropathy, Fig. 1 Light micrograph of early membranous nephropathy exhibiting normal architecture and peripheral capillary basement membranes of normal thickness. This light microscopic appearance in a patient with nephrotic syndrome can be indistinguishable from normal or minimal change disease (Silver methenamine; magnification X400)



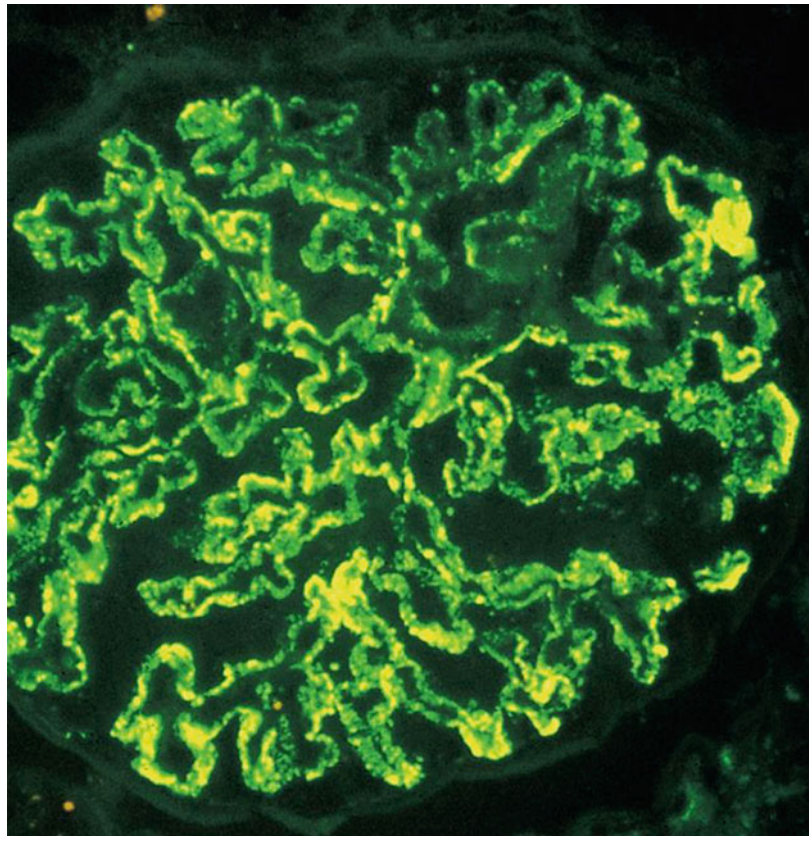
Translational Aspects of Membranous Nephropathy, Fig. 2 Morphologically advanced MN. There is a uniform increase in the thickness of the glomerular capillary walls throughout the glomerulus (Membranous change, *arrows*) without any increase in glomerular cellularity (Periodic acid–Schiff; magnification 400X)

have been identified by IF in the deposits in secondary MN including hepatitis B virus, nucleosomes in lupus MN, and tumor-associated proteins in MN associated with malignancy (Couser 2012).

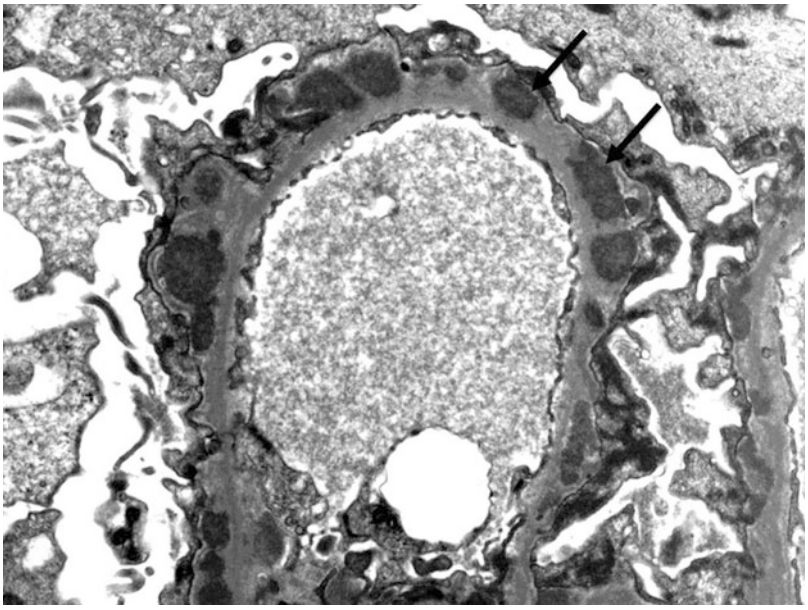
EM in IMN confirms the exclusively subepithelial localization of electron-dense deposits, which are often in slit, pore regions between adjacent podocyte foot processes (Fig. 5). GBM thickening is seen with progression, and the deposits gradually are incorporated within new GBM and then become more electron-lucent as they are resorbed before disappearing. This has led to classification of



Translational Aspects of Membranous Nephropathy, Fig. 3 Light microscopic appearance of a glomerulus in chronic IMN stained with silver methenamine to reveal the characteristic "spikes" projecting from the outer, subepithelial aspect of the basement membrane (*arrow*). Spikes are seen in more advanced disease (Magnification X1000)



Translational Aspects of Membranous Nephropathy, Fig. 4 Typical pattern of immunofluorescence staining for IgG in MN showing diffuse, finely granular deposits along the outer (subepithelial) aspect of the glomerular capillary wall. Similar staining can be seen with stains for C3, C5b-9, and, in active MN, for PLA2R (X400)



Translational Aspects of Membranous Nephropathy, Fig. 5 Low-power electron micrograph of MN showing typical discrete electron-dense deposits (*arrows*) along the outer (subepithelial) aspect of the glomerular capillary wall. Spikes of basement membrane can be seen projecting between deposits to form the spikes seen on silver staining. There is widespread effacement of overlying podocyte foot processes (X 18,000)

the EM changes into stages 1–4, which reflect the activity and chronicity of the disease (Couser and Cattran 2010a; Fervenza et al. 2008). Additional biopsy findings that are suggestive of secondary disease and rarely seen in IMN include electron-dense deposits in subendothelial or mesangial locations, significant mesangial cell proliferation, prominent deposition of IgG_1 and IgG_3, and tubuloreticular structures by EM. The other characteristic finding by EM is swelling and effacement, or "fusion," of podocyte foot processes, similar to findings in most proteinuric glomerular diseases that occur consequent to primary podocyte injury (Fig. 5) (Couser and Cattran 2010; Fervenza et al. 2008). With time there is detachment of damaged cells where protein flux becomes greatest and development of podocytopenia, another feature common to several chronic progressive proteinuric nephropathies.

In patients with persistent proteinuria and progressive disease, glomeruli eventually become scarred and sclerotic, and an interstitial mononuclear cell infiltrate develops between glomeruli followed by interstitial fibrosis as occurs in all chronic progressive glomerular diseases (Couser and Cattran 2010; Fervenza et al. 2008; Hladunewich et al. 2009).

Pathogenesis

The studies over a period of 50 years that have led to our current understanding of the pathogenetic mechanisms that underlie IMN represent a remarkable tribute to the value of basic, translational, and clinical research pursued by physician–scientists in finally unlocking the underlying autoimmune pathogenesis of an important human renal disease. They are well summarized elsewhere (Couser 2012; Beck and Salant 2010; Ronco and Debiec 2010). Attempts to clarify how the subepithelial immune deposits of IgG and complement form in MN began in the 1960s with models of acute and chronic serum sickness induced in rabbits immunized with bovine serum albumin (BSA). It was shown that chronic administration of native BSA sufficient to maintain a slight excess of antigen over antibody led to formation of low molecular weight, low avidity, and circulating soluble immune complexes coincident with the appearance of glomerular immune deposits of both antigen and antibody that were primarily subepithelial in distribution (Couser 1985). The deposits were believed to reflect the passive trapping of preformed immune complexes that penetrated to the subepithelial side of the glomerular filter to

precipitate beneath the podocyte layer (Couser 1985). However, in these BSA-serum sickness models, substantial deposit formation also occurred in mesangial and subendothelial locations, unlike the deposits in IMN, which were exclusively subepithelial. Moreover, subsequent studies were unable to verify that immune complexes of the proposed size were able to transit the capillary wall intact to deposit in the subepithelial space or that circulating immune complexes were even present in IMN in man (Couser 1985). Finally, other studies showed that subepithelial formation of BSA–anti-BSA immune deposits were independent of circulating immune complexes and depended largely on the electrical charge of the BSA antigen which appeared to localize independently of antibody and lead to subsequent "in situ" immune complex formation (Couser 1985).

Another model of MN had been described in 1959 by Walter Heymann who immunized rats with an antigenic preparation derived primarily from renal proximal tubular brush border that was later termed fraction 1A (Fx1A). Heymann found, over 4–6 months, that these rats developed a lesion that was virtually indistinguishable clinically and pathologically from IMN in man (active Heymann nephritis) (Couser 2012; Beck and Salant 2010; Kerjaschki 2004). Others later were able to induce the same deposits within days by injecting rabbit IgG antibodies to Fx1A (passive Heymann nephritis, PHN). However, these deposits were not accompanied by proteinuria until later in the disease when rat antibodies to the deposited rabbit IgG developed and a new process of deposit formation intervened. Based on the rabbit BSA-serum sickness studies, both the active and passive Heymann models were presumed to reflect chronic trapping of circulating immune complexes containing tubular antigens (Couser 1985). However, the mechanisms of tissue injury induced by immune deposit formation in glomeruli had only been defined in models of anti-GBM nephritis where complement activation, C5a chemotactic factor generation, and neutrophil recruitment were central (Couser 2012; 1985). They were unknown in traditional immune complex nephritis including the serum sickness or Heymann models where complement deposition also occurred but in the absence of any corresponding neutrophil infiltrate or inflammatory changes.

In the late 1970s, the PHN model was successfully modified to exhibit proteinuria that was induced by deposition of the injected heterologous anti-Fx1A IgG within 5 days (before the animals began to deposit a secondary antibody to the initial heterologous IgG) allowing the study of how the primary nephritogenic antibody caused glomerular injury without producing morphologic evidence of inflammation. This was eventually achieved with a high-titer sheep antibody to Fx1A that induced nephrotic-range proteinuria in rats within 3–4 days, a short enough interval to allow the mediators of glomerular injury to be defined. Two unexpected observations immediately followed. The first was that when the sheep anti-Fx1a antibody IgG used to induce proteinuric PHN was added to a non-recirculating, bloodless perfusate in a physiologic isolated perfused kidney system, within 1–2 h, it induced exactly the same deposits seen in 3–4 days following intravenous injection in the intact animal (Couser et al. 1978). This finding, reported almost simultaneously by Couser et al. and by the group led by Philip Hoedemaeker in the Netherlands using an ex vivo perfusion system, excluded any role for circulating immune complex trapping in producing the lesion of IMN and proved that the deposits instead formed in situ when antibody bound to some fixed glomerular antigen. Similar findings were obtained using rat antibody eluted from the kidneys of animals with active Heymann nephritis. The Heymann antigen, based on the exclusive localization of the deposits beneath podocyte foot processes, was hypothesized to be a constituent of the podocyte, and MN was therefore postulated to be an autoimmune disease (Couser et al. 1978; Couser 1985). Extensive later studies by Kerjaschki and Farquhar in the 1980s confirmed this and identified the antigen in Heymann nephritis as megalin, a normal constituent of the rat podocyte membrane (Kerjaschki 2004). However, megalin is not expressed in human podocytes. Another 25 years elapsed before it

was finally shown that IMN in man could result from the same mechanism involving in situ deposit formation by an anti-podocyte antibody as first in the Heymann models in rats.

The second unanticipated observation was that the injury (proteinuria) was entirely complement dependent despite the fact that no inflammatory cells were present thus excluding the only recognized role of complement in tissue injury – that is, attracting neutrophils via release of chemotactic C5a leading to neutrophil-mediated damage (Cybulsky 2011; Salant et al. 1980; Couser et al. 1985). It was speculated that this new role for complement might be due to glomerular formation of the complement membrane attack complex, C5b-9, which could insert into lipid bilayers of resident glomerular cells and activate these cells to release mediators such as oxidants and proteases that could, in turn, injure the capillary wall and cause proteinuria (Salant et al. 1980). This hypothesis was later confirmed ex vivo in isolated perfused kidneys using sheep anti-Fx1A IgG added to C6- and C8-deficient serum perfusate and in vivo using selective C6 depletion in the intact animal (Cybulsky 2011; Couser and Nangaku 2006). The initial demonstration that C5b-9 could mediate immune tissue injury was later extended to models of immune injury to both endothelial and mesangial cells as well and has since been verified in complement-mediated immune damage in several other disease states including neurons in multiple sclerosis, cardiac cells in myocardial infarcts, retinal epithelium in acute macular degeneration, and renal tubular cells in progressive proteinuric nephropathies of both immune and nonimmune origin (Cybulsky 2011).

Debiec and Ronco provided the first documentation of a fixed podocyte antigen causing IMN in humans in 2002 when they studied an infant with MN and nephrotic syndrome born to a mother with a genetic deficiency in neutral endopeptidase (NEP). They showed that NEP was expressed on podocytes of the infant and that the MN lesion resulted from alloimmunization with deposition of maternal anti-NEP antibody – which could also transfer MN to normal animals (Ronco and Debiec 2010). However, this mechanism proved applicable only to the alloimmunization setting and is not operative in typical adult IMN. However, in 2009, Beck and Salant utilized microdissection of glomeruli from patients with IMN, microelution of deposited antibody, and proteomic technology to demonstrate that the deposited IgG, primarily IgG4, in IMN was reactive with another podocyte membrane protein PLA2R (Beck et al. 2009). Moreover, 70–80 % of IMN patients studied had circulating anti-PLA2R antibody which not only correlated clinically with proteinuria but disappeared before remission and returned with relapse (Beck et al. 2009; Ronco and Debiec 2010; Hofstra et al. 2011). These findings, now confirmed by several other laboratories, bring full circle the observations made initially in the Heymann models of MN in rats to explain the pathogenesis of IMN in man.

In addition to membrane proteins NEP and PLA2R, antibodies to other podocyte antigens have also been identified in a minority of patients with IMN including cytosolic constituents such as superoxide dismutase, aldose reductase, and enolase, although the latter likely represent reactive secondary rather than primary disease-inducing antibodies.

Much has also been learned about the mechanism of C5b-9-mediated tissue injury that was first identified in PHN in 1980 (Cybulsky 2011). In contrast to antigen–antibody complexes which form lattices that are capped and shed from the podocyte to create the visible subepithelial deposits beneath the podocyte slit diaphragm, membrane insertion of C5b-9 is followed by uptake of the complex into multivesicular bodies within the cell and extrusion into the urinary space leading to elevated levels of urinary C5b-9 excretion in MN (Couser 2012; Couser and Nangaku 2006). Membrane insertion of C5b-9 in sublytic quantities has a potent agonistic effect on nucleated cells activating several signalling pathways and thereby converting normal glomerular cells to effector cells that produce mediator molecules such as oxidants, proteases, cytokines, growth factors, and several extracellular matrix components that lead (in the case of podocytes) to the membranous changes and spike

formation seen in MN (Cybulsky 2011). C5b-9 also alters cell-matrix interaction favoring detachment, podocyte shedding in the urine, and podocytopenia. It causes DNA damage that prevents completion of the cell cycle and therefore cell proliferation to repair the areas of denuded GBM created by podocyte detachment. The combination of oxidant injury to underlying GBM and podocyte detachment are believed to be the principal mechanisms that underlie the partial disruption of the glomerular barrier to protein filtration and consequent nonselective loss of large quantities of serum proteins in the urine in MN (Couser 2012; Cybulsky 2011).

Studies of models of secondary MN such as cationic BSA-induced chronic serum sickness or the autologous phase of PHN confirm that subepithelial deposits involving nonrenal antigens also form in situ and also cause injury through a C5b-9-dependent process (Couser 2012; Couser and Nangaku 2006).

A final important point in understanding the pathophysiology of capillary wall injury in MN is that sequestration of deposits in the subepithelial space and slow resorption by fully differentiated podocytes results in very delayed resolution of the disease after formation of new deposits ceases. This has been documented experimentally by transplanting MN kidneys (Heymann nephritis) into normal hosts and confirmed in man by the lag of weeks or months between the disappearance of anti-PLA2R antibody from the circulation and the eventual resolution of proteinuria (Hofstra et al. 2011).

Treatment

Any discussion of treatment of IMN must be prefaced with the reminder that less than 50 % of patients in most studies develop progressive disease and many patients need only general antiproteinuric supportive therapy since reducing urine protein excretion to less than 3.5 g/day is associated with a <5 % chance of progression (Couser and Cattran 2010; Fervenza et al. 2008). Thus, treatment to reduce edema with diuretics, correct hyperlipidemia with statins, and control blood pressure and lower protein excretion with renin–angiotensin system blockers titrated to achieve the lowest possible protein excretion is the standard of care for all patients with protein excretions exceeding 1 g/day (Feehally et al. 2012; Hofstra and Wetzels 2012). Studies have also shown that a conservative "watch and wait" approach during 6 months of conservative therapy does not alter the long-term outcome of the disease years later (although initial resolution of nephrotic syndrome is delayed by this approach, a consequence that is likely outweighed by sparing many patients the adverse consequences of immunosuppressive drug therapy) (Hofstra and Wetzels 2012). For patients who fail to achieve a 50 % reduction in protein excretion or have persistent proteinuria exceeding 3.5 g/day after 6 months of conservative therapy, patients who present initially with proteinuria >10 g/day, or patients exhibiting disabling nephrotic syndrome or progressive loss of renal function (evident by a 30 % increase in serum creatinine levels), the risk of progression exceeds 50 % and the addition of immunosuppressive therapy is warranted (Couser and Cattran 2010; Fervenza et al. 2008; Hofstra and Wetzels 2012). Disease-specific immunosuppressive therapy is not indicated in patients who have <3.5 g/day of proteinuria or have already lost substantial renal function – for example, those with eGFR <30 ml/min or serum creatinine >3.5 mg/dl (Feehally et al. 2012; Hofstra and Wetzels 2012).

The best established treatment regimen for IMN is to initiate steroid therapy with intravenous methylprednisolone (30 mg/kg/day to a maximum of 1,000 mg) for 3 days followed by 27 days of oral steroids (prednisone, 0.5 mg/kg not exceeding 60 mg/day) followed by a month of oral cyclophosphamide, 2–2.5 mg/day (adjusted for age and renal function) (Couser and Cattran 2010; Fervenza et al. 2008; Feehally et al. 2012). When this alternating monthly cycle of steroids and cytotoxic drugs is repeated for three cycles (6 months), remissions of proteinuria occur in about 70–80 % of patients vs. about 30 % of controls treated with supportive care only, and the development of ESRD 10 years later is reduced

from 30–40 % to 10 % or less when all patients with IMN are treated (Hofstra and Wetzels 2012; Hofstra and Wetzels 2010; Feehally et al. 2012). Comparable results have been reported at 7 years when the delayed therapy approach described above is employed (Hofstra and Wetzels 2012; Hofstra and Wetzels 2010). Observational studies suggest that treating for 6 months with daily oral cyclophosphamide and steroids achieves comparable results, although the cumulative dose of administered cyclophosphamide is twofold higher. The relapse rate following treatment with these regimens is about 25 %, and relapses are usually treated by repeating the same therapy that induced the initial remission (Feehally et al. 2012).

The only other treatment for MN established to improve long-term outcomes is calcineurin inhibitors (CNI), usually cyclosporine A (CSA), 3.5–5 mg/kg/day in divided doses to maintain blood levels of 120–200 μg/L, combined with low-dose steroids (prednisone 0.15 mg/kg/day) (Couser and Cattran 2010; Fervenza et al. 2008; Feehally et al. 2012). CSA has been shown to induce partial or complete remissions in 40–50 % of cases of IMN and should be employed if Cytoxan/steroid treatment fails, the patient cannot tolerate cytotoxic drugs, or issues such as preservation of fertility are operative (although MN is rarely progressive in young women). Results with Tacrolimus (.05 to .075 mg/kg/day, levels of 3–5 μg/L increasing to 5–8) are comparable, although the incidence of diabetes as a complication is higher. The relapse rate with CNIs is higher (40–50 %) than with cyclophosphamide (25 %) but seems to diminish with longer periods of therapy (Couser and Cattran 2010; Fervenza et al. 2008; Feehally et al. 2012). However, CNI nephrotoxicity is also a problem. Attempting to balance the nephrotoxicity with the increased relapse rate after short-term therapy, current recommendations are to use CNIs only in patients with normal or near-normal renal function (GFR $>$ 40 ml/min), to reduce initial doses every 4–8 weeks after achieving a complete or partial remission to 50 % of the initial dose, to treat for at least 1 year after a complete remission is achieved and 18 months after a partial remission, and to taper the drug slowly over several months (Couser and Cattran 2010; Fervenza et al. 2008; Feehally et al. 2012).

Less well-established treatment options for IMN include mycophenolate mofetil (MMF), B cell depletion with Rituximab and ACTH, and these may be employed in patients resistant to, or intolerant of, cyclophosphamide or CNIs. Pilot studies have suggested MMF, 2–3 g/day for 6 months, may give comparable short-term results to steroids and cyclophosphamide, at least in Asian patients. MMF is equivalent to cyclophosphamide for efficacy in membranous lupus nephritis, but long-term data on preservation of renal function are lacking. Rituximab used in a single dose of 375 mg/M2 i.v. has also been reported to reduce anti-PLA2R antibody levels and induce remission in about 50 % of patients in 1 year (Couser and Cattran 2010; Fervenza et al. 2008; Feehally et al. 2012; Hofstra et al. 2011), but that figure steadily increases to about 90 % by 6 years, and the safety profile is better than that of cyclophosphamide. ACTH therapy was shown to give short-term results equivalent to monthly cycles of cyclophosphamide and steroids with minimal adverse events in one small randomized controlled study but another controlled study comparing the two favored cyclophosphamide. MMF, rituximab, and ACTH all need to be compared to a cyclophosphamide/steroid protocol in properly designed prospective controlled trials with long-term follow-up before their places in the therapeutic armamentarium for IMN can be firmly established. Unfortunately, because of the indolent nature of the disease and slow rate of progression, this will take many years to accomplish by which time other more effective therapies may become available. Of particular potential with regard to IMN are newer B cell depleting drugs and complement inhibitors.

Renal transplantation is effective in patients who do reach ESRD from MN. The disease recurs in about 30 % of allografts and can alter allograft survival (Couser and Cattran 2010; Fervenza et al. 2008; Moroni et al. 2011). De novo MN is also a common cause of nephrotic syndrome in renal transplant recipients. Recurrent MN is associated with return of anti-PLA2R antibody

(Hofstra et al. 2011), but the mechanism of de novo MN in transplants does not involve anti-PLA2R and has not yet been defined. Employing cyclophosphamide as the primary immunosuppressive agent in transplant patients with MN is often recommended.

Treatment options for secondary MN depend on the etiology. Except when the disease occurs in association with other autoimmune diseases such as lupus, anti-GBM antibody disease, or ANCA-associated vasculitis, cytotoxic drug therapy is usually not indicated (Couser and Cattran 2010; Fervenza et al. 2008). Anti-PLA2R antibody is not generally detectable in cases of secondary MN including lupus although up to 30 % of patients with cancer-associated MN tested positive in one study. When MN is secondary to infections, exposure to drugs or toxins, and cancer, it is generally transient, resolves following successful treatment of the primary disease, and rarely progresses.

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Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Cancer and Nephrotic Syndrome
- ▶ Complement Regulation in the Kidney
- ▶ Environment and Autoimmunity
- ▶ Epigenetics in Autoimmunity
- ▶ Immune Responses to the Hepatitis C Virus
- ▶ Impact of Recurrent Autoimmune Diseases in Renal Transplant Outcomes
- ▶ Indications for Biopsy in Autoimmune GN
- ▶ Lupus Nephritis, Diagnosis and Treatment
- ▶ Paraneoplastic Neurological Syndromes, Overview
- ▶ TGF-β
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Beck Jr LH, Salant DJ. Membranous nephropathy: recent travels and new roads ahead. Kidney Int. 2010;77:765–70.

Beck Jr LH, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361:11–21.

Couser WG. Mechanisms of glomerular injury in immune complex disease. Kidney Int. 1985;28:569–83 (Nephrology Forum).

Couser WG. Mediation of immune glomerular diseases. Basic and translational concepts. J Am Soc Nephrol. 2012;23(3):381–99. doi:10.1681/ASN.2011030304. (Published online before print January 26, 2012).

Couser WG, Cattran D. Membranous nephropathy. Chapter 22. In: Floege J, Johnson RJ, Feehally J, editors. Comprehensive clinical nephrology. 4th ed. St Louis: Saunders; 2010. p. 248–59.

Couser WG, Nangaku M. Cellular and molecular biology of membranous nephropathy. J Nephrol. 2006;19:699–705.

Couser WG, Steinmuller DR, Stilmant MM, Salant DJ, Lowenstein LM. Experimental glomerulonephritis in the isolated perfused rat kidney. J Clin Invest. 1978;62:1275–87.

Couser WG, Baker PJ, Adler S. Complement and the direct mediation of immune glomerular injury: a new perspective. Kidney Int. 1985;28:879–900 (Editorial review).

Cybulsky AV. Membranous nephropathy. Contrib Nephrol. 2011;169:107–25.

Feehally JF, Cattran DC, et al. KDIGO clinical practice guidelines for glomerulonephritis. Kidney Int. 2012;82(8):840–56. doi:10.1038/ki.2012.280.

Fervenza FC, Sethi S, Specks U. Idiopathic membranous nephropathy: diagnosis and treatment. Clin J Am Soc Nephrol. 2008;3:905–19.

Hladunewich MA, Troyanov S, Calafati J, Cattran DC. Metropolitan Toronto glomerulonephritis registry. The natural history of the non-nephrotic membranous nephropathy patient. Clin J Am Soc Nephrol. 2009;4:1417–22.

Hofstra JM, Wetzels JF. Alkylating agents in membranous nephropathy: efficacy proven beyond doubt. Nephrol Dial Transplant. 2010;6:1760–6.

Hofstra JM, Wetzels JF. Management of patients with membranous nephropathy. Nephrol Dial Transplant. 2012;27:7–9.

Hofstra JM, Beck Jr LH, Beck DM, Wetzels JF, Salant DJ. Anti-phospholipase A_2 receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. Clin J Am Soc Nephrol. 2011;6:1286–91.

Jefferson AJ, Couser WG. Membranous nephropathy in the pediatric population. Chapter 59. In: Avner E, Harmon W, Niaudet P, Yoshikawa N, editors. Pediatric nephrology. 6th ed. Berlin/eidelberg: Springer; 2009. p. 799–814.

Kanigicherla D, Gummadova J, McKenzie EA, Roberts SA, Harris S, Nikam M, Poulton K, McWilliam L, Short CD, Venning M, Brenchley PE. Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. Kidney Int. 2013. doi:10.1038/ki.2012.486. [Epub ahead of print].

Kerjaschki D. Pathomechanisms and molecular basis of membranous glomerulopathy. Lancet. 2004;364:1194–6.

Moroni G, Gallelli B, Quaglini S, et al. Long-term outcome of renal transplantation in patients with idiopathic membranous glomerulonephritis. Nephrol Dial Transplant. 2011;25:3408–15.

Ronco P, Debiec H. Membranous glomerulopathy: the evolving story. Curr Opin Nephrol Hypertens. 2010;19:254–9.

Salant DJ, Belok S, Madaio MP, Couser WG. A new role for complement in experimental membranous nephropathy in rats. J Clin Invest. 1980;66:1339–50.

Stanescu HC, Arcos-Burgos M, Medlar A, Bockenhauer D, Kottgen A, Dragomirescu L, Voinescu C, Patel N, Pearce K, Hubank M, Stephens HA, Laundy V, Padmanabhan S, Zawadzka A, Hofstra JM, Coenen MJ, den Heijer M, Kiemeney LA, Bacq-Daian D, Stengel B, Powis SH, Brenchley P, Feehally J, Rees AJ, Debiec H, Wetzels JF, Ronco P, Mathieson PW, Kleta R. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. N Engl J Med. 2011;364:616–26.

Transplantation for Autoimmune Liver Diseases

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Synonyms

Autoimmune Hepatitis: Chronic Active Hepatitis; Hepatocellular Carcinoma: Hepatoma, Liver Cell Cancer; Primary Biliary Cirrhosis: Chronic Nonsuppurative Destructive Cholangitis; Pruritus: Itching

Definition

The most common autoimmune liver diseases that may lead to liver transplantation are autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC).

Indications for Liver Transplantation: The indications and contraindications for liver transplantation (LT) in autoimmune liver diseases are broadly similar as for other liver diseases. LT may be required to treat patients with end-stage liver disease or to treat intractable symptoms that make the patient's quality of life unacceptable (Carbone and Neuberger 2011). Although survival after transplantation for autoimmune liver disease is very good, with those grafted for PBC having some of the best outcomes, neither the quality nor the length of life is normal. Survival is reduced by many factors, including an increased risk of cardiovascular disease, some malignancies, some infections, recurrent disease, and the specific effects of immunosuppressive agents, such as diabetes and renal impairment (Li and Neuberger 2009).

However, most patients would expect 5-year survival in excess of 85 %.

For those with end-stage disease, a survival benefit of transplantation becomes apparent when the MELD (model for end-stage liver disease) score exceeds 15 (Carbone and Neuberger 2011). Of course, models such as MELD have their limitations, but they do provide an invaluable tool to guide management and an objective basis for allocating donated organs. Those who develop liver cell cancer (hepatocellular carcinoma (HCC)) may also be candidates for transplantation and most centers use the Milan criteria, although this restriction remains controversial.

Other indications for transplantation include intractable encephalopathy and intractable pruritus. Options for treatment of itching include cholestyramine, rifampicin, naltrexone, and even treatment with plasmapheresis or MARS (molecular absorbent recycling system). Lethargy is not an indication for transplantation since while fatigue scores may improve after transplantation, they rarely return to normal. Management should focus on the exclusion of treatable causes of lethargy (such as undiagnosed Addison's disease, thyroid disease, or the impact of inappropriate use of antihistamines to treat pruritus); modafinil

has been used but is not licensed in this indication. The presence of cholangiocarcinoma will usually contraindicate transplantation as recurrence is high although encouraging outcomes are seen in highly selected patients who may follow a regime with brachytherapy and chemotherapy (Ilyas et al. 2011).

Outcomes After Transplantation: Outcomes are usually excellent, but recurrence remains a significant factor affecting both the survival of graft and patient.

Recurrence of PBC: The diagnosis of recurrent PBC (rPBC) is made on the basis of histology, with demonstration of the characteristic nonsuppurative destructive cholangitis. Antimitochondrial antibody (AMA) titers usually fall transiently after transplantation but then rise (Silveira et al. 2010). The subtypes usually remain unchanged. Extrahepatic associated disease associated with PBC (such as thyroid disease or celiac disease) may become apparent after transplant. Liver tests may show a cholestatic pattern, but histological evidence of recurrence may be seen in the presence of normal liver tests. The reported rates of recurrent PBC vary, primarily because of variations in the diagnosis and the use of protocol liver biopsies. Reported rates of recurrence are around 30–40 % at 5 years. Recurrence may lead to graft cirrhosis, but graft loss from recurrence is low (about 4 %). There are few identified risk factors for recurrence: use of ciclosporin is associated with less common and less severe recurrence although no impact of choice of immunosuppressive agents on outcomes has yet been shown. Some reports have suggested that lack of corticosteroids might be associated with recurrence (Silveira et al. 2010). The role of genetic factors on recurrence has yet to be clarified.

There is no effective treatment for recurrence: use of ursodeoxycholic acid (UDCA) 10–15 mg/kg/day is associated with improved liver tests, but no impact has been shown on graft or patient survival or progression of graft damage.

Recurrence of PSC: The diagnosis of recurrent PSC (rPSC) is more complex, and differentiation between rPSC and secondary sclerosing cholangitis is sometimes difficult. The diagnosis of rPSC is made on the basis of multiple non-anastomotic biliary strictures in the absence of other factors: factors that may be associated with non-anastomotic strictures include rejection, graft/host ABO incompatibility, ischemic, hepatic artery thrombosis, and infection (bacterial and viral). Histological features in the graft may also make the diagnosis (Fosby et al. 2012; Carbone and Neuberger 2011). The demonstration of multiple non-anastomotic strictures is commonly made on biliary imaging by magnetic resonance imaging: most patients with PSC have a Roux-en-y biliary drainage procedure as the bile duct is often affected by the disease, and cholangiocarcinoma may develop in the remnant after transplant; as a consequence, endoscopic demonstration of biliary strictures is difficult although percutaneous biliary imaging may be effective. It is unusual to find single dominant strictures; so biliary dilatation is rarely effective.

Treatment is Uncertain: As indicated above, biliary dilatation is technically difficult and rarely effective and is associated with cholangitis. UDCA (10–15 mg/kg/day) may improve liver tests, but no impact on graft survival has been shown. UDCA is not licensed for use in this indication.

As with rPBC, reported rates of recurrence are variable and may reach 50 % at 5 years; unlike rPBC, rPSC may lead to graft loss in 20 % at 5 years. For those few who have had a regraft for rPSC, disease recurrence is variable.

Several studies have assessed risk factors (Fosby et al. 2012): our own study has shown that colectomy either before or during transplantation protects against recurrence, but this has yet to be confirmed in other studies. Other factors that are associated with rPBC include acute rejection and CMV infection.

Most patients with PSC have inflammatory bowel disease affecting the colon, usually ulcerative colitis. The course of colitis is variable (Fosby et al. 2012), with approximately one third improving, one third remaining static, and one third deteriorating. There are no reliable factors that predict the outcome of the colon. All liver transplant recipients have an increased risk of colonic polyps and neoplasm, but this risk is significantly greater in those with colitis. Most centers advocate the

use of annual colonoscopy and a low threshold for surgical intervention; this approach seems sensible but is not evidenced based.

Recurrence of Autoimmune Hepatitis: The diagnosis of recurrent autoimmune hepatitis (rAIH) is based on a combination of factors: transplantation for AIH, high titers of autoantibodies (antinuclear antibodies (ANA) > 1:100 or liver-kidney microsomal antibodies, elevated immunoglobulins (IgG > 1.5 times upper limit of normal), elevated transaminases), and liver histology showing interface hepatitis in the absence of other factors such as rejection and viral infection (Duclos-Vallee and Sebagh 2009; Mendes et al. 2011). A similar condition may be seen in those who were transplanted for conditions other than AIH, and this has been termed de novo AIH (Liberal et al. 2012): it remains uncertain whether this condition truly represents a new syndrome, development of AIH or is just a form of modified rejection. Histological features of rAIH may be found in the presence of normal liver tests and, again, the use of protocol biopsies recommended.

The Risk Factors for rAIH Are Uncertain: The impact of donor and recipient HLA is conflicting. The treatment of rAIH is with the introduction or increase amount of corticosteroids, but fibrosis may progress despite treatment. Use of mycophenolate may also be of help. Most centers advocate the long-term use of corticosteroids in these patients, using doses such as prednisolone 5–10 mg/day, with bone protection measures.

Living Donation and Recurrent Disease: Numbers of patients receiving living donor grafts for autoimmune disease are small, but some studies suggest that recurrence may be more common in such patients (Duclos-Vallee and Sebagh 2009; Ilyas et al. 2011).

Cross-References

- ▶ Immunosuppression in Clinical Liver Transplantation
- ▶ Impact of Recurrent Autoimmune Diseases in Renal Transplant Outcomes
- ▶ Primary Biliary Cirrhosis, Overview
- ▶ Primary Sclerosing Cholangitis: Clinical and Systemic Manifestations and Treatment
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Carbone M, Neuberger J. Liver transplantation in PBC and PSC: indications and disease recurrence. Clin Res Hepatol Gastroenterol. 2011;35:446–54.

Duclos-Vallee JC, Sebagh M. Recurrence of autoimmune disease, primary sclerosing cholangitis, primary biliary cirrhosis and autoimmune hepatitis after liver transplantation. Liver Transpl. 2009;15(suppl 2):S25–34.

Fosby B, Karlsen TH, Melum E. Recurrence and rejection in liver transplantation for primary sclerosing cholangitis. Worls J Gastroenterol. 2012;18:1–15.

Ilyas JA, O'Mahony CA, Vierling JM. Liver transplantation in autoimmune liver diseases. Best Pract Res Clin Gastroenterol. 2011;25:765–82.

Li KK, Neuberger J. Recurrent nonviral disease following liver transplantation. Expert Rev Gastroenterol Hepatol. 2009;3:257–68.

Liberal R, Longhi MS, Grant CR, Mieli-Vergani G, Vergani D. Autoimmune hepatitis after liver transplantation. Clin Gastroenterol Hepatol. 2012;10:346–53.

Mendes F, Couto CA, Levy C. Recurrent and de novo autoimmune liver diseases. Clin Liver Dis. 2011;15:859–78.

Silveira MG, Talwalkar JA, Lindor KD, Wiesner RH. Recurrent biliary cirrhosis after liver transplantation. Am J Transplant. 2010;10:720–6.

Tregs in the Liver

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Synonyms

Regulatory T cells; Treg

Definition

Regulatory T cells (Treg) (Bregs:IL-10) are important mediators of immune tolerance to

self-antigens or alloantigens, and their impairment may cause autoimmune disease (Sakaguchi et al. 2008). However, the regulatory function of Treg can also limit the immune responses toward pathogens or cancer cells. Regulatory T cells can control immune responses by downmodulating activation and effector functions of various cell types including CD4 and CD8 T cells, B cells, NK cells, NKT cells, and dendritic cells. Several Treg subsets can be differentiated by their phenotype and suppressive mechanisms (Jonuleit and Schmitt 2003). Among these subsets, CD4 + CD25 + Foxp3+ Treg are the most abundant and best characterized Treg population, and their fundamental role in controlling immune responses has been described in various contexts such as autoimmunity, transplantation, and cancer (Sakaguchi et al. 2008). These CD4 + CD25 + Foxp3+ Treg exert their suppressive function mainly through cell contact by membrane-bound molecules but also contact independently through secreted molecules, such as transforming growth factor-beta (TGF-β), interleukin-10, or interleukin-35 (Shevach 2009). The majority of the CD4 + CD25 + Foxp3+ Treg are generated in the thymus; however, these cells may also be generated in the periphery, either by expansion of thymic Treg or by TGFβ-dependent de novo conversion from Foxp3-negative nonregulatory CD4+ T cells (Curotto de Lafaille and Lafaille 2009). Most of the data on CD4 + CD25 + Foxp3+ Treg has been generated in mice, in which these Treg cells can be easily identified by their Foxp3 expression. Characterization of CD4 + CD25 + Foxp + Treg in humans is more difficult, and unambiguous distinction from activated nonregulatory effector T cells requires detection of additional parameters, such as a low expression level of CD127 (Liu et al. 2006). Other Treg subsets are Tr1 cells (Treg Subsets: TR1) and Th3 cells that suppress through contact-independent secretion of interleukin-10 (Treg Subsets: IL-10) or TGF-β, respectively (Jonuleit and Schmitt 2003). Besides the secretion of these cytokines, there are no defined markers that specifically identify Tr1 or Th3 cells. Of note, all activated Treg cells of whatever subset exert their suppressive activity in a nonspecific way, affecting in particular those cells that are in close proximity; however, the activation of Treg cells depends on the specific recognition of cognate antigen through their T-cell receptor.

Treg and Healthy Liver

The liver is increasingly being recognized as an immunological organ that can establish multiple interactions with circulating T cells under the relatively slow blood flow in the sinusoidal network (Racanelli and Rehermann 2006). In the healthy liver, such interactions most often result in the induction of a state of immune tolerance (Carambia and Herkel 2010). The generation of Treg cells through stimulation by liver cells is of central importance for the establishment of hepatic immune tolerance (Thomson and Knolle 2010) (▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance). Two major mechanisms seem to account for the hepatic generation of Treg cells: (1) the interleukin 10-dependent generation of Tr1-like cells (Thomson and Knolle 2010) and (2) the TGFβ-dependent generation of CD4 + CD25 + Foxp3+ Treg cells (Lüth et al. 2008). Indeed, both interleukin-10 and TGF-β are expressed in the liver by various cells types and in relative abundance (Carambia and Herkel 2010; Thomson and Knolle 2010). The capacity of the liver to induce Treg is currently being explored for use in induction of specific tolerance to gene products through hepatic gene transfer (LoDuca et al. 2009).

Treg and Autoimmune Liver Disease

Since Treg have the capacity to control autoimmune responses, it is conceivable that a functional or numerical impairment of Treg may contribute to the pathogenesis of autoimmune liver diseases. Indeed, global Treg defects have been reported for patients with autoimmune hepatitis or primary biliary cirrhosis (reviewed in

Czaja and Manns 2010). However, these findings should be interpreted with caution, since the majority of these studies have not been performed with unambiguous Treg markers, leaving the possibility that activated nonregulatory T cells had been assessed. Thus far, the analysis of antigen-specific Treg that recognize relevant autoantigens has been far more difficult, partially because only a few disease-related antigens have been identified (Czaja and Manns 2010) and partially because the antigen-specific Treg frequency in blood is very low (Longhi et al. 2011). Nevertheless, functional antigen-specific Treg can be expanded from the blood of patients (Longhi et al. 2011). Therefore, expanded antigen-specific Tregs may enable specific immunotherapies for autoimmune liver disease.

Treg and Viral Hepatitis

Treg are believed to contribute to the impaired immune responses to virus in patients with chronic infection with hepatitis B virus (HBV) (▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response) or hepatitis C virus (HCV) (▶ Immune Responses to the Hepatitis C Virus) (Rehermann 2007). Indeed, several studies (reviewed in Manigold and Racanelli 2007) indicate that both chronic HBV and chronic HCV infection is associated with increased Treg frequencies in peripheral blood. As most of these studies have not been performed with unambiguous Treg markers, these findings should be interpreted with caution. However, several reports of increased intrahepatic frequencies of viral antigen-specific Treg in chronic viral infection (reviewed in Carambia and Herkel 2010) support the idea that Treg may contribute to impaired antiviral immunity. Moreover, several groups reported a functional suppression of virus-specific T cells by Treg from chronically infected patients (in Carambia and Herkel 2010). Therefore, functional inhibition or depletion of virus-specific Treg may facilitate viral clearance and resolution of hepatitis. However, there is thus far no methodology for the selective targeting of antigen-specific Treg cells. Currently, it seems only possible to inhibit or deplete all Treg irrespective of their specificity; it remains to be seen whether such treatment option is safe and effective.

Treg and Liver Cancer

Although effector T-cell responses to various tumor-associated antigens can be detected in patients suffering from hepatocellular carcinoma (reviewed in Breous and Thimme 2011), these T-cell responses most often cannot effectively deplete the malignant cells. Treg, which are part of the lymphocytic tumor infiltrate, are considered to be a major reason for the poor effectiveness of anticancer immunity (Breous and Thimme 2011). Indeed, pharmaceutical Treg impairment was able to unmask a CD4+ T-cell response to tumor-associated antigen (Greten et al. 2010). Thus, it is conceivable that T-cell-based immunotherapies for the treatment of liver cancer could greatly benefit from combining with a functional inhibition of tumor-associated Treg. However, as mentioned above, it is currently not possible to selectively target antigen-specific Treg, and it is not clear whether a transient nonspecific Treg impairment is both safe and effective.

Conclusions

The suppression of immune responses by Treg serves tissue protection through prevention of autoimmune disease, or through limiting excessive immunity to pathogens. However, the suppressor activity of Treg can also contribute to tissue damage by preventing efficient clearance of pathogens or eradication of malignant cells. Therefore, both, activation and inhibition of Treg activity, have great potential for therapeutic use. Several approaches are currently being explored that can increase or decrease Treg frequency or function. However, to date the development of Treg-based therapies is significantly limited by (1) the lack of suitable methodologies for the selective manipulation of Treg

specifically recognizing defined antigens and (2) the lack of defined antigens that drive pathogenic (auto-) immune responses.

Cross-References

- ▶ Acute and Chronic Hepatitis B Virus Infection, Immune Response
- ▶ Immune Responses to the Hepatitis C Virus
- ▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance

References

Breous E, Thimme R. Potential of immunotherapy for hepatocellular carcinoma. J Hepatol. 2011;54:830–4.
Carambia A, Herkel J. CD4 T cells in hepatic immune tolerance. J Autoimmun. 2010;34:23–8.
Czaja AJ, Manns MP. Advances in the diagnosis, pathogenesis, and management of autoimmune hepatitis. Gastroenterology. 2010;139:58–72. e4.
de Lafaille MA C, Lafaille JJ. Natural and adaptive Foxp3 + regulatory T cells: more of the same or a division of labor? Immunity. 2009;30:626–35.
Greten TF, Ormandy LA, Fikuart A, Höchst B, Henschen S, Hörning M, Manns MP, Korangy F. Low-dose cyclophosphamide treatment impairs regulatory T cells and unmasks AFP-specific CD4+ T-cell responses in patients with advanced HCC. J Immunother. 2010;33:211–8.
Jonuleit H, Schmitt E. The regulatory T cell family: distinct subsets and their interrelations. J Immunol. 2003;171:6323–7.
Liu W, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med. 2006;203:1701–11.
LoDuca PA, Hoffman BE, Herzog RW. Hepatic gene transfer as a means of tolerance induction to transgene products. Curr Gene Ther. 2009;9:104–14.
Longhi MS, Hussain MJ, Kwok WW, Mieli-Vergani G, Ma Y, Vergani D. Autoantigen-specific regulatory T cells, a potential tool for immune-tolerance reconstitution in type-2 autoimmune hepatitis. Hepatology. 2011;53:536–47.
Lüth S, Huber S, Schramm C, Buch T, Zander S, Stadelmann C, Brück W, Wraith DC, Herkel J, Lohse AW. Ectopic expression of neural autoantigen in mouse liver suppresses experimental autoimmune neuroinflammation by inducing antigen-specific Tregs. J Clin Invest. 2008;118:3403–10.
Manigold T, Racanelli V. T-cell regulation by CD4 regulatory T cells during hepatitis B and C virus infections: facts and controversies. Lancet Infect Dis. 2007;7:804–13.
Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology. 2006;43:S54–62.
Rehermann B. Chronic infections with hepatotropic viruses: mechanisms of impairment of cellular immune responses. Semin Liver Dis. 2007;27:152–60.
Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;133:775–87.
Shevach EM. Mechanisms of Foxp3+ T regulatory cell-mediated suppression. Immunity. 2009;30:636–45.
Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol. 2010;10:753–66.

Tumor Macrophages

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Synonyms

Glioma-infiltrating macrophages; TIMs; Tumor-infiltrating macrophages/microglia

Definition

Inflammatory cells of monocytic lineage present in the neoplastic environment of central nervous system glial tumors.

Basic Characteristics

Introduction

Glioblastoma is a high-grade glial tumor that has remained one of the deadliest human malignancies despite decades of basic scientific and clinical research. In the United States alone, this disease is diagnosed in approximately 10,000 patients per year, and the median survival of newly diagnosed glioblastoma following surgery, radiation, and chemotherapy is about 1 year (Louis et al. 2007). Particularly as new therapeutic avenues are sought, there has been increasing interest in

leveraging the patient's own innate and adaptive immune responses in the fight against glial tumors.

The central nervous system (CNS) has a unique relationship with the immune system that sets it apart from all other organ systems in the body. Notable features include the blood-brain barrier comprises tight endothelial junctions and pericapillary astrocytic processes, the absence of conventional lymphatic vessels, and the relative paucity of major histocompatibility complex expression and antigen-presenting cells. While it would seem that these properties inevitably impact the manner in which the immune system responds to tumors arising in the CNS, many of these classically recognized CNS-specific immunological features may themselves change under neoplastic conditions. Central players in the immune response to glial tumors are both tumor-infiltrating macrophages/microglia (TIMs) and tumor-infiltrating lymphocytes (TILs). The role of TIMs will be discussed in this entry, while a separate entry will review the role of TILs in the immunobiology of glioblastomas.

Microglia, a Mesenchymal Glial Cell of Innate Immunity

Microglia are mesenchymal, hematopoietic-derived glial cells that are the resident mononuclear phagocytes of the CNS. They serve in part as the innate immune system's frontline defense and also play a key role in activating the adaptive immune system. In their quiescent state, their morphology is characterized by ramified processes. In response to infection or injury, microglia change their phenotype into an activated state; they become ameboid in shape, similar to peripherally derived activated macrophages, and they upregulate proteins important in mediating a variety of immune responses. Chief among these are increased expression of MHC class II and costimulatory proteins that aid microglia in their capacity to perform as APCs. Additionally, microglia release proinflammatory cytokines and chemokines, such as TNF alpha (Graeber et al. 2002).

As of yet there is no single specific marker to distinguish microglia from peripherally derived macrophages. Some groups have used the relative expression levels of CD45 as a way to distinguish two subpopulations of CD11b-expressing cells, and there is evidence to suggest that $CD11b^{+}$ $CD45^{low}$ cells are in fact microglia, while a $CD11b^{+}$ $CD45^{high}$ immunophenotype marks other, peripherally derived, macrophages (Badie and Schartner 2001). However, it may be that different activation states impart different immunophenotypes on a given cell type, and such uncertainties have made definitive microglial identification in the context of complex immunobiological environments as yet controversial. Furthermore, the differential expression of CD45 mentioned above was conducted in experiments with rodents, and other groups have not reproduced this difference in human tissue (Hussain et al. 2006).

The Role of Microglia in Glioma Immunobiology

There is evidence that TIMs constitute a large fraction of leukocytes found in glioma tissue, and, by some reports, a large fraction of all cells in these tumors. For example, experiments using flow cytometry with human tissue show that an average of 0.825 % of all cells in resected GBM tissue comprise microglia/macrophages (CD45, CD11b+, CD11c+) versus 0.007 % in normal brain (Hussain et al. 2006). By comparison, in the same study, CD4+ T cells accounted for 0.099 % of cells, and CD8+ T cells accounted for 0.039 % of cells. Other groups, using immunohistochemical methods and manual cell counting have found as many as 30.1 % of glioblastoma cells to be CD11b+, with an average of 10.4 % over 5 nonrecurrent tumor samples (Morimura et al. 1990). Even higher numbers were obtained using other macrophage markers. Certainly, while it is clear that TIMs are indeed present, further analysis is required to definitively quantitate their extent, with particular attention being paid to the manner in which these cells are defined by their immunophenotype and/or other criteria.

Recent data shows that the principle effect of TIMs may be to aid the progression of tumors rather than initiate an immune attack. It has been

shown that microglia may be co-opted by glioma cells to mediate an immunosuppressive effect. For example, driven by glioma cell release of cytokines such as TGF beta, TIMs upregulate B7-H1, which causes programmed cell death of nearby T effector cells. In vitro assays have also shown an upregulation of IL-10 and FASL in TIMs, which suppresses cytotoxic T cell responses (Badie and Schartner 2001). At the same time, proinflammatory cytokines such as TNF alpha are downregulated in microglia when in the presence of glioma cells. Finally, TIMs engage in a number of other activities beneficial to tumorigenesis such as the degradation of extracellular protein networks which facilitates tumor cell migration, as well as the secretion of vascular and tumor cell growth factors, such as epidermal growth factor (EGF) (Graeber et al. 2002; Yang et al. 2010). EGF is known to play an important role in the progression of glioblastomas, and its receptor is frequently both mutated and amplified in primary glioblastomas (Chin et al. 2008).

Therapeutic Promise of TIMs

The majority of current evidence suggests that TIMs are accomplices of the glioma cell and that glioblastomas have recruited them to promote neoplastic disease progression. Is it hopeless, therefore, to pursue these immunological players as potential therapeutic mediators? Current experimental evidence is mixed in that strategies employing both TIM activation and suppression have shown improved survival in animal models of glioma. Injections of CpG oligodeoxynucleotide, which activate TIMs via a toll-like receptor pathway, improve animal survival (Carpentier et al. 2003). At the same time, other groups have shown that the suppression of TIMs, for example, via inhibition of STAT3, can also slow the progression of tumors (Zhang et al. 2009). What seems clear, however, is that manipulating TIMs in some fashion may prove to be an important adjunctive therapeutic modality. However, the best method for utilizing these cells, whether to attack or to stifle malignant gliomas, remains to be elucidated.

Cross-References

▶ Tumor-Infiltrating T Cells

References

Badie B, Schartner J. Role of microglia in glioma biology. Microsc Res Tech. 2001;54:106–13.

Carpentier AF, Auf G, Delattre JY. CpG-oligonucleotides for cancer immunotherapy: review of the literature and potential applications in malignant glioma. Front Biosci. 2003;8:e115–27.

Chin L, Meyerson M, et al. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008; 455:1061–1068.

Graeber MB, Scheithauer BW, Kreutzberg GW. Microglia in brain tumors. Glia. 2002;40:252–9.

Hussain SF, Yang D, Suki D, Aldape K, Grimm E, Heimberger AB. The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. Neuro Oncol. 2006;8:261–79.

Louis DN, Ohgaki H, Wiestler OD, Cavanee WK (2007) WHO classification of tumours of the central nervous system. Lyon, France: International Agency for Research on Cancer (IARC); vol 4th edn.

Morimura T, Neuchrist C, Kitz K, Budka H, Scheiner O, Kraft D, Lassmann H. Monocyte subpopulations in human gliomas: expression of Fc and complement receptors and correlation with tumor proliferation. Acta Neuropathol. 1990;80:287–94.

Yang I, Han SJ, Kaur G, Crane C, Parsa AT. The role of microglia in central nervous system immunity and glioma immunology. J Clin Neurosci. 2010;17:6–10.

Zhang L, Alizadeh D, Van Handel M, Kortylewski M, Yu H, Badie B. Stat3 inhibition activates tumor macrophages and abrogates glioma growth in mice. Glia. 2009;57:1458–67.

Tumor-Infiltrating T Cells

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Synonyms

Glioma-infiltrating lymphocytes; Glioma-infiltrating T cells; TILsp; Tumor-infiltrating lymphocytes

Definition

Lymphocytes with expression of T cell markers present in the neoplastic environment of central nervous system glial tumors.

Basic Characteristics

A Diversity of T Cells in the Tumor Microenvironment

Studies have shown that a diversity of inflammatory cell types is present within high-grade gliomas. A basic introduction to the role of monocytic derived cells in the glioma environment is presented in a separate entry (see "► Tumor Macrophages"). In this entry, we focus on T cells in the context of glioma biology. As will be discussed below, T cells exhibiting a variety of immunophenotypes can be found within glioma tissue, although their relative quantities vary depending on the study. Despite the presence of both potential cytotoxic and humoral mediators, however, there is a clear inability of the host to mount an effective response to high-grade glial neoplasms. The numerous factors contributing to this failure have only begun to be understood, and the ability to boost or suppress specific T cell functions in a therapeutic context depends upon a deeper understanding of the role of individual T cell types in the glioma environment.

Tumor Antigen Delivery to T Cells

The basic steps required for an intrinsic cytotoxic T cell response to gliomas include revelation of tumor antigen by neoplastic cells, retrieval and processing of antigen by antigen-presenting cells (APCs); travel of APCs to, and communication with, naive T cells at sites including those likely remote from the tumor itself; travel of T cells back to the site of the tumor; and, finally, activation and persistence of T cell function within the tumor environment. Efforts to reveal the APCs relevant to the endogenous glioma response have pointed to both dendritic cells and macrophage/microglial cells as potentially important players (Dunn et al. 2007). Experimental evidence suggests that intraparenchymal CNS antigens are successfully delivered to cervical lymph nodes by APCs where they interact with T cells able to home to CNS capillaries and into the parenchymal bed. However, the identification of glioma tumor antigens that could potentially initiate a potent T cell effector response requires further investigation. Certainly, antigens that are relatively glioma-specific have been identified and include a variant of the epidermal growth factor receptor (EGFRvIII) that results from an in-frame deletion and is seen in a relatively high percentage of de novo glioblastomas (Dietrich et al. 2010). Other antigens of interest include SART3, whose expression is restricted to gliomas in limited datasets, and IL13Rα2, whose expression profile in normal tissue remains to be clarified (Dunn et al. 2007). Of course, if such antigens are to be employed in the context of immune therapy, their specificity becomes crucial in limiting toxicity to nonneoplastic brain parenchyma.

T Cell Subtypes and Their Role in Gliomas

Experimental evidence has shown that multiple T cell classes are present within the glioma environment. These include CD4+, CD8+, and CD4+ CD25+ cells. The latter immunophenotype, especially when also FOXP3+, is thought to be a marker for T regulatory cells (Tregs) which are of particular interest due to their role as potent immunosuppresive agents (Shevach 2002). The relative proportion of these different cell types varies considerably among different studies. For example, one group that used flow cytometry to characterize the lymphocyte population of 10 glioblastoma resection specimens reported that 17.7 % of cells were lymphocytes. Of these, 25 % were CD3+, and of those, 18.9 % were CD4+ CD25- while 6.2 % were CD4+ CD25+ (El Andaloussi and Lesniak 2006). Studies conducted in the 1980s utilized variable quantitative methodologies. One study that presorted cells with both gross dissection and Percoll gradient centrifugation followed by immunohistochemical labeling found that CD45+ cells accounted for a range of 11–67 % of tumor cells in 4 high-grade gliomas; of these, approximately 42 % were CD4+ cells and 41 % were CD8+ cells

(Farmer et al. 1989). Other studies conducted around the same time used semi-quantitative methods to estimate the extent of lymphocytic infiltration (i.e., slight, moderate, or intense) (von Hanwehr et al. 1984). Clearly, more robust and standardized methods to accurately capture the diversity of lymphocytes present in high-grade gliomas are needed. Adding to the complexity is the variation in human samples themselves, for example, did the patient undergo a previous resection, irradiation, or treatment with immunomodulatory agents? All of these interventions likely have an impact on the immune system players present in a given specimen.

As the relentless progression of virtually all high-grade gliomas would suggest, the ability of the adaptive immune system to mount an effective response is limited. Animal models with spontaneous glioma formation show both a lymphocytic and monocytic response relatively early in the progression of tumors, long before the animals become symptomatic (such analyses are unfortunately limited in the context of human high-grade gliomas that are rarely found incidentally prior to the onset of symptoms). The composition of these infiltrates does indeed include helper cells, cytotoxic cells, and Tregs. The exact role of this adaptive response with respect to tumor evolution remains incompletely understood. Critically, the degree to which an ineffective initial immune response encourages the formation of less antigenic tumor subclones, and the degree to which Tregs generate an immunosuppressive environment are essential areas of ongoing investigation (Tran Thang et al. 2010).

Prognostic Implications of TILs

While it is not unusual to see a lymphocytic infiltrate in high-grade glial tumors and within specimens, this feature is heterogeneous across specimens and often times it is virtually absent. For example, the lymphocytic infiltrate may be robust and perivascular, it may be present diffusely throughout the tumor in smaller numbers, or it may be difficult to appreciate without immunohistochemical aides. The question of whether the presence of TILs itself is of prognostic relevance has been addressed in several studies. Early studies found an increase in survival on the order of months in patients with high-grade gliomas that showed significant perivascular cuffing of lymphocytes (Brooks et al. 1978; Dunn et al. 2007). Subsequent studies have also found positive correlations with survival, whereas others have found either no correlation or even negative correlations (Dunn et al. 2007).

Therapeutic Considerations

Leveraging the adaptive immune system in the treatment of high-grade gliomas is of great interest, especially as tumor-specific antigens become better characterized. Two strategies that will be briefly discussed are active vaccination immunotherapy and adoptive T cell therapy.

Vaccination strategies that employ cultured dendritic cells or utilize direct injection of tumor-specific peptides to induce humoral- and/or delayed-type hypersensitivity immune responses have already been tested in early phase clinical trials (Choi et al. 2009). Specifically, a peptide derived from the EGFRvIII protein described above has been used in vaccination trials that show some promise in extending tumor-free progression with limited toxicity in small numbers of patients (Choi et al. 2009). However, further trials including randomized studies are needed to confirm such benefits. Of concern however is that tumors initially showing definitive expression of EGFRvIII may lose such expression in the face of immunotherapy, with subsequent evasion of any mounted immune response. Alternatively, it may be that the tumor itself initially has heterogeneous expression of a given epitope, generating a survival advantage for those cells lacking expression of the targeted epitope in the context of vaccination. Such cancer immunoediting likely will necessitate the administration of multidimensional therapies that simultaneously attack several tumor antigens, modulate the tumor population to enrich for cells bearing the targeted epitope, and/or alter the tumor microenvironment to increase the efficacy of a given immune response.

Regarding adoptive T cell therapy, numerous strategies for introducing populations of cytotoxic T cells that have been primed and expanded ex vivo

have been tested. Sources for these T cells have varied from autologous memory T cells obtained from the peripheral blood to TILs obtained from the tumor bed itself. The key theoretical benefits of such adoptive strategies in comparison to active immunotherapy is the ability to generate higher percentages of tumor antigen-directed T cells and the ability to manipulate and optimize the T cell phenotype in culture prior to patient introduction (Mitchell et al. 2003). An oft-cited trial utilizing in vitro expansion of autologous tumor bed-derived TILs showed a partial or complete response in three of six patients after 2 years (Quattrocchi et al. 1999). This study, while encouraging, lacked enough power to make strong conclusions about therapeutic efficacy; nevertheless the protocol appeared safe and the relatively low dose of IL-2 used adjunctively appeared well tolerated. More studies are clearly needed with sufficient numbers of patients to account for differences in adjunctive therapies, recurrence status, time since surgery, and the baseline immune status of the patient, among other factors.

Cross-References

► Tumor Macrophages

References

Brooks WH, Markesbery WR, Gupta GD, Roszman TL. Relationship of lymphocyte invasion and survival of brain tumor patients. Ann Neurol. 1978;4:219–24.

Choi BD, Archer GE, Mitchell DA, Heimberger AB, McLendon RE, Bigner DD, Sampson JH. EGFRvIII-targeted vaccination therapy of malignant glioma. Brain Pathol. 2009;19:713–23.

Dietrich PY, Dutoit V, Tran Thang NN, Walker PR. T-cell immunotherapy for malignant glioma: toward a combined approach. Curr Opin Oncol. 2010;22:604–10.

Dunn GP, Dunn IF, Curry WT. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human glioma. Cancer Immun. 2007;7:12.

El Andaloussi A, Lesniak MS. An increase in CD4+ CD25+ FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. Neuro Oncol. 2006;8:234–43.

Farmer JP, Antel JP, Freedman M, Cashman NR, Rode H, Villemure JG. Characterization of lymphoid cells isolated from human gliomas. J Neurosurg. 1989;71:528–33.

Mitchell DA, Fecci PE, Sampson JH. Adoptive immunotherapy for malignant glioma. Cancer J. 2003;9:157–66.

Quattrocchi KB, Miller CH, Cush S, Bernard SA, Dull ST, Smith M, Gudeman S, Varia MA. Pilot study of local autologous tumor infiltrating lymphocytes for the treatment of recurrent malignant gliomas. J Neurooncol. 1999;45:141–57.

Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol. 2002;2:389–400.

Tran Thang NN, Derouazi M, Philippin G, Arcidiaco S, Di Berardino-Besson W, Masson F, Hoepner S, Riccadonna C, Burkhardt K, Guha A, et al. Immune infiltration of spontaneous mouse astrocytomas is dominated by immunosuppressive cells from early stages of tumor development. Cancer Res. 2010;70:4829–39.

von Hanwehr RI, Hofman FM, Taylor CR, Apuzzo ML. Mononuclear lymphoid populations infiltrating the microenvironment of primary CNS tumors. Characterization of cell subsets with monoclonal antibodies. J Neurosurg. 1984;60:1138–47.

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Ultrastructure of the Liver Sinusoid

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The Liver Sinusoid

The liver sinusoids are the blood vessels that make up the microcirculation of the liver. Ultrastructure refers to the morphological cellular features that can be resolved by electron microscopy.

The liver has many immunological roles including production of acute-phase proteins, clearance of waste products generated by systemic inflammation and immune responses, deletion of activated T cells, and induction of tolerance to ingested and self-antigens (Parker and Picut 2012). The liver sinusoids form the interface between circulating blood and the hepatocytes thus are strategically placed to undertake and/or regulate the immune functions of the liver.

The sinusoidal vessels branch off from the terminal portal venules, carry blood between the plates of hepatocytes, and then drain into the central venules (Fig. 1). Each sinusoid traverses a distance of about 1 mm, and there are about one billion sinusoids in a human liver (Fraser et al. 2012). They occupy about 30 % of the liver volume and are a very complex network of vessels with a fractal dimension exceeding two (Warren et al. 2008). The periportal (zone I) sinusoids are more reticular in distribution and become more aligned or anisotropic as they move towards the pericentral (zone III) regions. The sinusoids are only 9–10 μm in diameter (Warren et al. 2008); therefore, blood flow is occasionally interrupted by circulating blood cells that have become blocked within the sinusoids. Moreover leukocytes and lymphocytes force plasma into the extracellular space as they squeeze through the sinusoids – a process termed "forced sieving" (Wisse et al. 1985).

The sinusoids contain four main cell types:

1. Liver sinusoidal endothelial cells (LSECs), which form the endothelial lining of the sinusoids and represent about 70 % of the total population of sinusoidal cells.
2. Kupffer cells, which are the resident macrophages of the liver and occupy the lumen of the sinusoids. They make up about 20 % of the sinusoidal cells but over 80 % of all fixed macrophages in the body.
3. Stellate cells, which are pericytes that lie within the extracellular space between the LSECs and hepatocytes. They are also known as fat-storing cells or Ito cells and represent less than 10 % of the sinusoidal cells.

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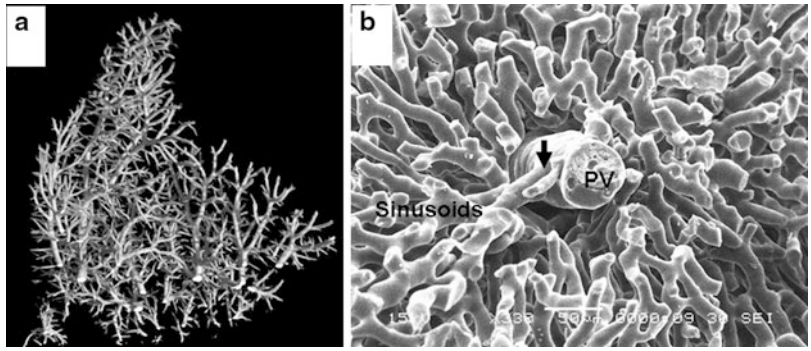


Ultrastructure of the Liver Sinusoid, Fig. 1 Vascular casts of the liver microcirculation. (**a**) 3D image of micro-CT scan showing the branching of the portal vein in one entire liver lobe. (**b**) Scanning electron micrograph of the network of sinusoidal vessels branching off (*arrow*) from a terminal portal venule (*PV*) (Source: Biogerontology Laboratory of the ANZAC Research Institute by Victoria Cogger, Alessandra Warren and Jennifer O'Reilly)

4. Pit cells, which are natural killer lymphocytes that reside in the sinusoidal lumen and make up less than 1 % of all sinusoidal cells.

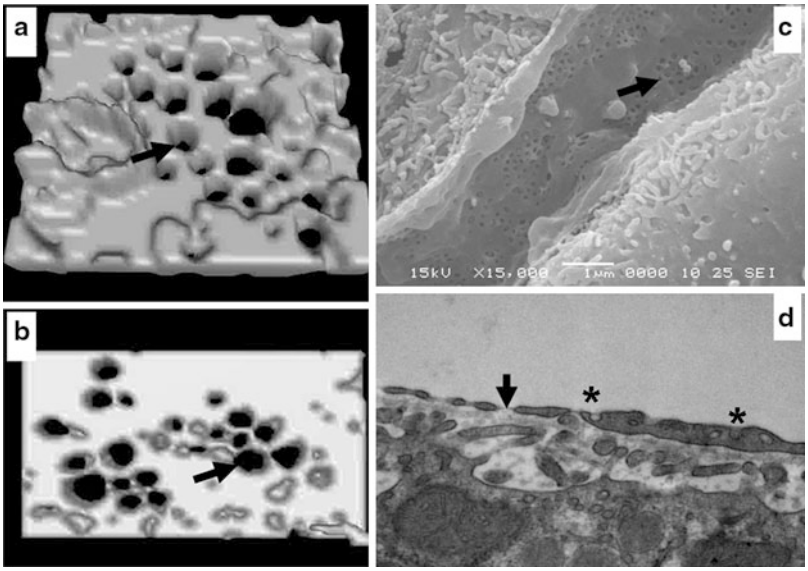
Liver Sinusoidal Endothelial Cells

LSECs are the endothelial cells of the hepatic sinusoids. LSECs are very thin and perforated with fenestrations that characterize the ultrastructure of the LSEC. Fenestrations are transcellular pores 50–150 nm in diameter, which occupy up to 20 % of the cell membrane surface of the LSEC (Fig. 2). These pores are not bridged by a diaphragm nor is there any underlying basement membrane in healthy livers; therefore, fenestrations provide unimpeded access of plasma in the sinusoid to the extracellular space (termed the space of Disse). About three quarters of fenestrations are found within clusters containing scores of fenestrations that have been called sieve plates because of their sieve-like appearance. A single-cultured LSEC may have many tens of sieve plates, representing perhaps many thousands of fenestrations per cell (Cogger and Le Couteur 2009).

Fenestrations cannot be resolved using standard light microscopic methods; therefore, they have been visualized primarily using transmission and scanning electron microscopy. Structured illumination light microscopy (SIM) can also visualize fenestrations and sieve plates in isolated rat LSECs without any of the preparation techniques required for electron microscopy (Cogger et al. 2010). This method confirmed that fenestrations are about 100 nm in diameter (123 ± 24 nm) with 26 ± 14 fenestrations in each sieve plate (Fig. 2).

Fenestrations are dynamic structures. The size and number of fenestrations have been influenced by various vasoactive hormones, cytokines, pharmaceutical agents, and physiological factors such as portal pressure, liver regeneration, and nutritional status. In particular, vascular endothelial growth factor (VEGF) and substances that disrupt the actin cytoskeleton increase the number and/or diameter of fenestrations (Cogger and Le Couteur 2009). Fenestrations are reduced in chronic liver disease, diabetes mellitus, and old age, while large gaps develop following acute toxic insults such as oxidative stress and acetaminophen poisoning (Cogger et al. 2004; Le Couteur et al. 2005).

The major physiological role of fenestrations appears to be the transfer of substrates between hepatocytes and the sinusoidal blood and hepatocytes. This includes particulate substrates with diameters less than that of the fenestrations (e.g., lipoproteins) or soluble substrates (Fraser et al. 2012). The loss of fenestrations that occurs in chronic liver disease and aging provides a mechanism contributing to the impairment of hepatic clearance seen in these conditions (Le Couteur et al. 2002, 2005).



Ultrastructure of the Liver Sinusoid, Fig. 2 Liver sinusoidal endothelial cells. (**a** and **b**) Three dimensional structured illumination micrographs of a sieve plate with fenestrations (*arrows*). (**c**) Scanning electron micrograph of a sinusoid perforated with fenestrations. (**d**) Transmission electron micrograph of liver sinusoidal endothelial cell showing fenestrations (*arrow*) and clathrin coated pits (*asterix*) (Source: Biogerontology Laboratory of the ANZAC Research Institute by Victoria Cogger, Alessandra Warren and Jennifer O'Reilly)

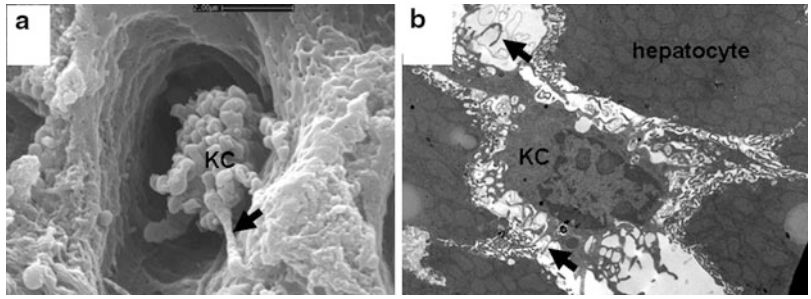
Fenestrations may also have a role in the ability of the liver to induce immunological tolerance. Hepatocytes have been shown to be tolerogenic antigen-presenting cells (Bertolino 2008; Holz et al. 2010). Electron microscopy revealed that resident lymphocytes and circulating naïve CD8+ T cells make direct contact with hepatocytes via fenestrations, a process termed trans-endothelial hepatocyte lymphocyte interaction (TEHLI) (Warren et al. 2006). Thus, fenestrations might regulate the first step in the development of immune tolerance by hepatocytes.

Electron microscopy also reveals numerous caveolae and clathrin-coated pits on both luminal and abluminal membranes of LSECs (Fig. 2d). These underpin the key role of the LSEC in endocytosis. The LSEC is responsible for the clearance of most blood-borne waste macromolecules including soluble IgG immune complexes, bacterial CpG, heparin, collagen and procollagen products, advanced glycation end products, chondroitin, acetylated LDLs, and hyaluronan. The LSEC carries pattern-recognition endocytosis receptors (mannose receptor and stabilin1/2) and the endocytic Fc gamma receptor IIb2 combined with a highly efficient endocytic machinery (Sørensen et al. 2012).

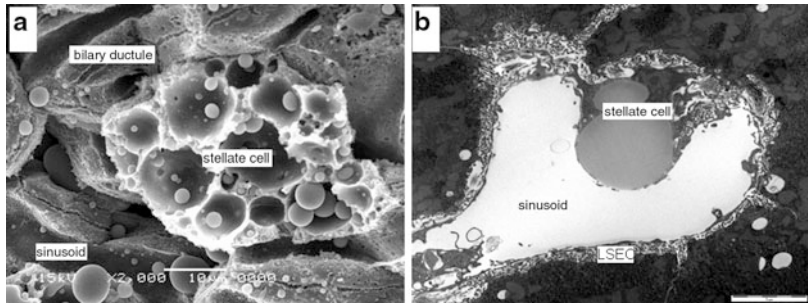
Kupffer Cells

The Kupffer cells are the major fixed macrophages in the body. Both Kupffer cells and LSECs form the scavenger cell system ("dual cell principle") with LSECs representing the professional pinocyte, clearing the blood of waste colloids and soluble macromolecules, while the Kupffer cell represents the professional phagocyte, eliminating larger particles including cells and bacteria (Sørensen et al. 2012). The Kupffer cell also produces cytokines such as TNF-α and interleukin-6 in response to inflammatory stimuli (Arii and Imamura 2000; Naito et al. 2004).

Kupffer cells are large ameboid cells that adhere to the luminal surface of the LSECs (Fig. 3). There are many more Kupffer cells in



Ultrastructure of the Liver Sinusoid, Fig. 3 Kupffer cells. (**a**) Scanning electron micrograph of Kupffer cell (*KC*) in the lumen of the sinusoid showing a lamellipodia extending to the LSEC (*arrow*). (**b**) Transmission electron micrograph of an activated Kupffer cells showing numerous lamellipodia (Source: Biogerontology Laboratory of the ANZAC Research Institute by Victoria Cogger, Alessandra Warren and Jennifer O'Reilly)



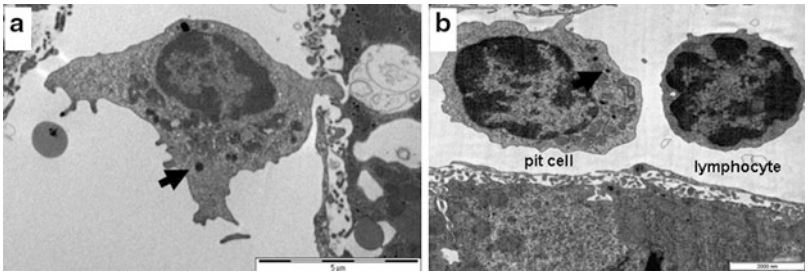
Ultrastructure of the Liver Sinusoid, Fig. 4 Stellate cell. (**a**) Scanning electron micrograph of a stellate cell engorged with lipid droplets. (**b**) Transmission electron micrograph showing stellate cell protruding into the lumen of the sinusoid (Source: Biogerontology Laboratory of the ANZAC Research Institute by Victoria Cogger, Alessandra Warren and Jennifer O'Reilly)

the periportal region of the sinusoid than the pericentral region. They have numerous projections (lamellipodia, microvilli), many of which attach to the LSECs and even extend through fenestrations into the extravascular space. Kupffer cells and their lamellipodia increase in number and size after activation. The cytoplasm contains large numbers of endocytic vesicles and lysosomes which also increase with Kupffer cell activation (Naito et al. 2004).

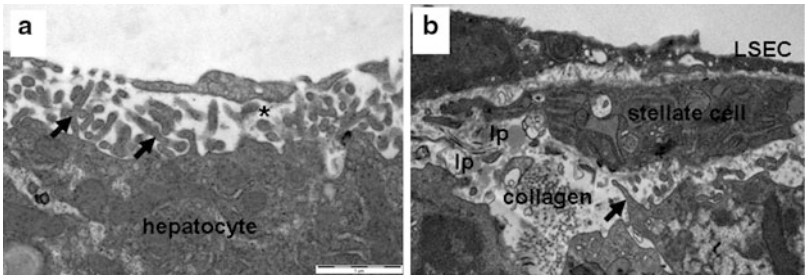
The role of Kupffer cells has been studied by depleting the liver of Kupffer cells with agents such as gadolinium chloride or clodronate liposomes. These studies have shown that Kupffer cell activation is a pivotal mechanism for many conditions including ischemia reperfusion injury, acetaminophen toxicity, and fatty liver. With aging there is an increase in basally active Kupffer cells; however, these do not respond effectively to inflammatory stimuli (Hilmer et al. 2007).

Stellate Cells

Hepatic stellate cells are pericytes that lie in the extracellular space between the LSEC and hepatocytes termed the space of Disse. They are spindle or star-shaped cells with long cytoplasmic extensions that surround the sinusoids (Fig. 4). The characteristic structural features of stellate cells are large lipid droplets containing vitamin A and triglycerides. In fact, over 80 % of vitamin A in the body is stored in stellate cells as retinyl palmitate, and this generates a distinctive autofluorescence (Senoo 2004; Senoo et al. 2007).



Ultrastructure of the Liver Sinusoid, Fig. 5 (**a**) Transmission electron micrograph of a large granular lymphocyte in the sinusoidal lumen containing electron dense vesicles in the cytoplasm consistent with pits (*arrow*). (**b**) Pit cell and lymphocyte in the sinusoid (Source: Biogerontology Laboratory of the ANZAC Research Institute by Victoria Cogger, Alessandra Warren and Jennifer O'Reilly)



Ultrastructure of the Liver Sinusoid, Fig. 6 Electron micrographs of the space of Disse. (**a**) Normal space of Disse containing hepatocyte microvilli (*arrow*) and extracellular matrix (*asterix*). (**b**) Space of Disse containing lipoproteins (*lp*) and collagen (Source: Biogerontology Laboratory of the ANZAC Research Institute by Victoria Cogger, Alessandra Warren and Jennifer O'Reilly)

In various liver diseases such as hepatic cirrhosis and fibrosis, stellate cells become activated. This is associated with loss of the lipid droplets and the development of a myofibroblastic appearance. There is increased rough endoplasmic reticulum and Golgi apparatus associated with increased production of extracellular matrix substances such as collagen (Senoo et al. 2007). On the other hand, old age is associated with increased numbers of nonactivated stellate cells that are swollen with large lipid droplets (Warren et al. 2011).

Pit Cells

Pit cells are large granular lymphocytes found in the lumen of the sinusoid (Fig. 5). The characteristic ultrastructural feature is the presence of "pits" or spherical dense granules about 200 nm in diameter within their cytoplasm that contain perforin and granzymes. They also contain rod-cored vesicles and tend to have kidney-shaped nuclei. Functionally, pit cells are natural killer cells (NK) with cytotoxic activity against tumor cells which provides protection against liver metastases. Natural killer T cells (NKT) are ultrastructurally similar to pit cells but also express the T cell receptor on their cell membrane (Nakatani et al. 2004; Parker and Picut 2012).

Space of Disse

The space of Disse is the extracellular space that lies between the LSEC and the surrounding hepatocytes (Fig. 6). The space contains extracellular matrix proteins and microvilli that project from the hepatocytes, some of which span the entire space of Disse and extend through fenestrations

into the lumen of the sinusoid. Plasma enters the space of Disse via fenestrations and flows in a retrograde direction towards the lymphatic vessels in the periportal regions to form hepatic lymph. Unlike other extravascular spaces, the space of Disse does not contain significant amounts of basal lamina, although continuous basal membranes develop in old age and various liver diseases (Le Couteur et al. 2005). There are only occasional collagen bundles which increase markedly in liver disease and diabetes mellitus. Sometimes smaller lipoproteins are seen in the space of Disse (Fraser et al. 2012). In some forms of hepatitis, inflammatory cells such as neutrophils enter the space of Disse via fenestrations (Warren et al. 2007). Peliosis refers to the situation where erythrocytes are found in the space of Disse in conditions such as heart failure and acetaminophen toxicity.

Cross-References

- ▶ Adaptive Immune Cells in the Liver
- ▶ Liver Sinusoidal Endothelial Cells: Role in Immunity and Tolerance
- ▶ Liver Vasculature and Microvasculature

References

Arii S, Imamura M. Physiological role of sinusoidal endothelial cells and Kupffer cells and their implication in the pathogenesis of liver injury. J Hepatobiliary Pancreat Surg. 2000;7:40–8.

Bertolino P. Impaired function of dendritic cells translocating the liver sinusoids: a veto effect contributing to intrahepatic tolerance? Eur J Immunol. 2008;38:938–41.

Cogger VC, Le Couteur DG. Fenestrations in the liver sinusoidal endothelial cell. In: Arias I, Wolkoff A, Boyer J, Shafritz D, Fausto N, Alter H, Cohen A, editors. The liver: biology and pathobiology. Hokoben: Wiley; 2009. p. 387–404.

Cogger VC, Muller M, Fraser R, McLean AJ, Khan J, Le Couteur DG. The effects of oxidative stress on the liver sieve. J Hepatol. 2004;41:370–6.

Cogger VC, McNerney GP, Nyunt T, Deleve LD, McCourt P, Smedsrod B, et al. Three-dimensional structured illumination microscopy of liver sinusoidal endothelial cell fenestrations. J Struct Biol. 2010;171:382–8.

Fraser R, Cogger VC, Dobbs B, Jamieson HA, Warren A, Hilmer SN, et al. The liver sieve and atherosclerosis. Pathology. 2012;44:181–6.

Hilmer SN, Cogger VC, Le Couteur DG. Basal activity of Kupffer cells increases with old age. J Gerontol A Biol Sci Med Sci. 2007;62:973–8.

Holz LE, Warren A, Le Couteur DG, Bowen DG, Bertolino P. CD8+ T cell tolerance following antigen recognition on hepatocytes. J Autoimmun. 2010;34:15–22.

Le Couteur DG, Fraser R, Cogger VC, McLean AJ. Hepatic pseudocapillarisation and atherosclerosis in ageing. Lancet. 2002;359:1612–5.

Le Couteur DG, Fraser R, Hilmer S, Rivory LP, McLean AJ. The hepatic sinusoid in aging and cirrhosis – effects on hepatic substrate disposition and drug clearance. Clin Pharmacokinet. 2005;44:187–200.

Naito M, Hasegawa G, Ebe Y, Yamamoto T. Differentiation and function of Kupffer cells. Med Electron Microsc. 2004;37:16–28.

Nakatani K, Kaneda K, Seki S, Nakajima Y. Pit cells as liver-associated natural killer cells: morphology and function. Med Electron Microsc. 2004;37:29–36.

Parker GA, Picut CA. Immune functioning in non lymphoid organs: the liver. Toxicol Pathol. 2012;40:237–47.

Senoo H. Structure and function of hepatic stellate cells. Med Electron Microsc. 2004;37:3–15.

Senoo H, Kojima N, Sato M. Vitamin A-storing cells (stellate cells). Vitam Horm. 2007;75:131–59.

Sørensen KK, McCourt P, Berg T, Crossley C, Le Couteur D, Wake K, et al. The scavenger endothelial cell – a new player in homeostasis and immunity. Am J Physiol. 2012;303:R1217.

Warren A, Le Couteur DG, Fraser R, Bowen DG, McCaughan GW, Bertolino P. T lymphocytes interact with hepatocytes through fenestrations in murine liver sinusoidal endothelial cells. Hepatology. 2006;44:1182–90.

Warren A, Bertolino P, Benseler V, Fraser R, McCaughan GW, Le Couteur DG. Marked changes of the hepatic sinusoid in a transgenic mouse model of acute immune-mediated hepatitis. J Hepatol. 2007;46:239–46.

Warren A, Chaberek S, Ostrowski K, Cogger VC, Hilmer SN, McCuskey RS, et al. Effects of old age on vascular complexity and dispersion of the hepatic sinusoidal network. Microcirculation. 2008;15:191–202.

Warren A, Cogger VC, Fraser R, Deleve LD, McCuskey RS, Le Couteur DG. The effects of old age on hepatic stellate cells. Curr Gerontol Geriatr Res. 2011;2011:439835.

Wisse W, De Zanger RB, Charels K, Van Der Smissen P, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Hepatology. 1985;5:683–92.

V

Vasculitis and the Kidney

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Synonyms

Renal

Definition

Vasculitis is a pathological process defined by leukocyte invasion and fibrinoid necrosis of the vessel wall. Any size vessel can be affected, and vasculitis may be associated with thrombotic occlusion, stenosis, or aneurysmal dilatation of the blood vessel.

Introduction

Systemic vasculitis occurs either as a primary autoimmune disorder or as a secondary manifestation of another disease process (Tables 1 and 2). Primary systemic vasculitis is classified according to the predominant size of vessel involved and the presence of circulating antineutrophil cytoplasmic autoantibodies (ANCA) (Fig. 1) (Jennette 2011; Watts et al. 2011). Small vessel vasculitides, especially those associated with ANCA, often involve the kidneys. Less commonly, large vessel vasculitides such as polyarteritis nodosa and Takayasu arteritis can involve the kidneys.

ANCA-associated vasculitis (AAV) is a relatively rare systemic disease, but accounts for 50 % of all systemic vasculitis presentations. The major AAV subgroups are granulomatosis with polyangiitis (GPA) (formerly called Wegener's granulomatosis) and microscopic polyangiitis (MPA).There is increasing evidence that ANCA is associated with the disease pathogenesis and that the two ANCA subtypes, proteinase 3-ANCA (PR3-ANCA) and myeloperoxidase-ANCA (MPO-ANCA), define clinical subgroups that differ in their survival, renal and cardiovascular outcomes, and renal histology.

AAV has a predilection for the kidney with more than 75 % of patients having renal involvement. These patients are at risk of progressing to end-stage renal failure if not treated promptly. The clinical manifestation of small vessel vasculitis in the kidney is often a rapidly progressive (crescentic) glomerulonephritis. This results from a glomerular capillaritis leading to a segmental, necrotizing glomerulonephritis with epithelioid crescent formation. For those with AAV, immune deposits are typically minimal or absent (pauci-immune) as opposed to Henoch-Schönlein purpura or cryoglobulinemia where immune deposits are present (Jennette et al. 1994).

In the past, untreated systemic vasculitis was often fatal, with only 20 % of patients survived at 1 year. The combination of cyclophosphamide and corticosteroid induces remission in up to 90 % of patients. Nevertheless, the survival of

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Vasculitis and the Kidney, Table 1 Primary systemic vasculitis

Predominant large vessel
Giant-cell arteritis
Takayasu arteritis
Predominant medium vessel vasculitis
Polyarteritis nodosa
Kawasaki
Predominant small vessel vasculitis
Granulomatosis with polyangiitis (Wegener's)
Microscopic polyangiitis
Churg-Strauss syndrome
Primary immune complex mediated
Henoch-Schönlein purpura
Cryoglobulinemia

Vasculitis and the Kidney, Table 2 Secondary causes of vasculitis

Infections	
Hepatitis B, C	HIV
Bacterial endocarditis	Mycobacteria
Syphilis	
Drugs	
Propylthiouracil	Allopurinol
Hydralazine	Thiazides
Penicillin	Sulphonamides
Malignancy (paraneoplastic)	
Myeloproliferative	
Lymphoproliferative	
Solid organ tumors	
Connective tissue disorders/autoimmune	
Lupus	Sarcoidosis
Rheumatoid arthritis	Sjogren's syndrome
Inflammatory dowel disease	Scleroderma
Poly/ Dermatomyositis	Antiphospholipid antibody syndrome
Others	
Essential cryoglobulinemia	Hypocomplementemic urticarial vasculitis
Illicit drugs	
Cocaine	

AAV patients is still worse compared to age- and sex-matched controls. The age of many patients at diagnosis, late presentations with advanced renal failure, histological severity, and the toxicity of current drugs contribute to poor outcomes, with ESRD or death occurring in 17 % of AAV patients at 1 year.

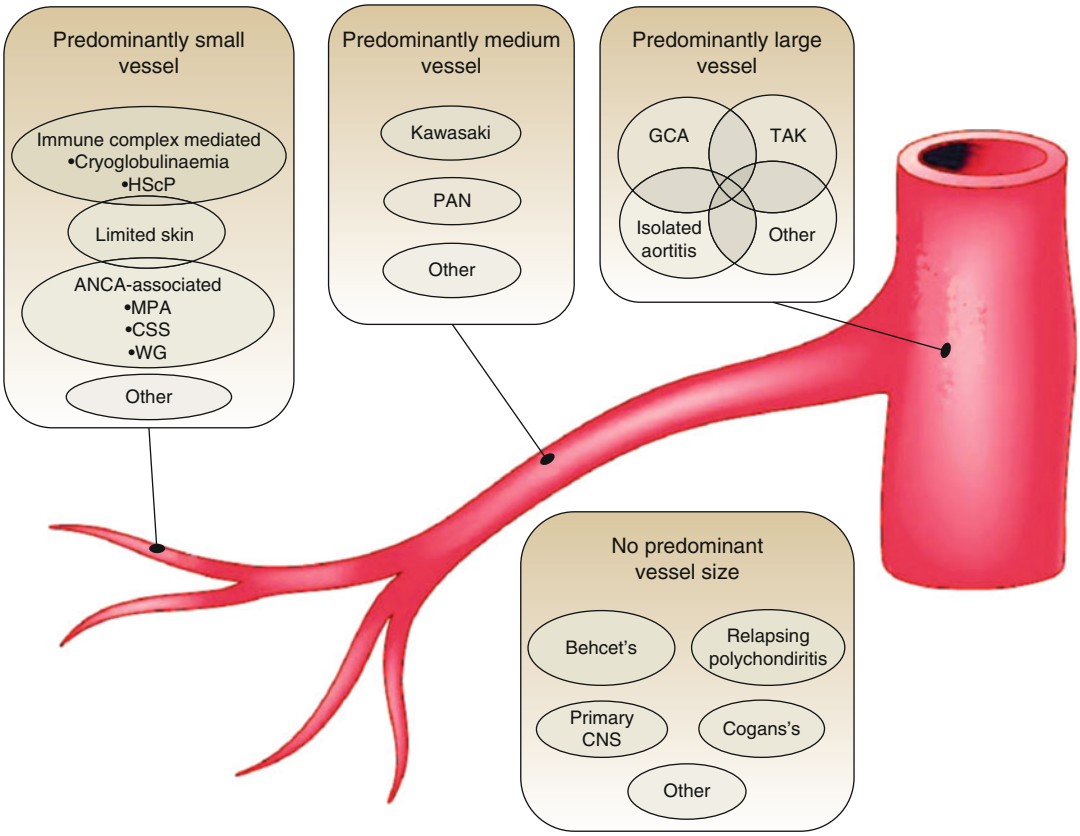
AAV is now characterized by a chronic relapsing and remitting course, with 50 % of patients relapsing within 5 years. Since the 1980s, there has been a growing awareness of the late toxicity of cyclophosphamide that includes infertility, bladder, and hematological malignancies. The advent of newer therapies, especially rituximab, as well as the use of adjunctive therapies such as plasma exchange and intravenous methylprednisolone has improved outcomes and reduced the need for cyclophosphamide.

Nomenclature, Definitions, and Classification of Primary Systemic Vasculitis

Primary systemic vasculitis syndromes were initially described as discrete clinicopathological entities: Henoch-Schönlein purpura in 1837, polyarteritis nodosa in 1866, Takayasu arteritis in 1910, microscopic polyangiitis in 1923, Wegener's granulomatosis (GPA) in 1936, and Churg-Strauss angiitis in 1951. An International Consensus on the terminology of vasculitis syndromes resulted in the 1992 Chapel Hill statement (Table 3), which forms the basis for current classification systems. The availability of ANCA testing, especially with modern solid-phase assays, has been a major step in the diagnosis and monitoring of vasculitis and has provided insights into pathogenesis and classification (Savige et al. 1999).

Epidemiology and Demography of AAV

The incidence of primary systemic vasculitis is 40/million/year with AAV comprising 15–20/million/year. An apparent increased incidence of AAV has been explained by improved detection, especially in the elderly, but where long-term epidemiology studies have been performed, no increase in incidence has been seen. Prevalence rates of AAV range from 90 to 400/million (Lane et al. 2005). Although the



Vasculitis and the Kidney, Fig. 1 Revised draft classification scheme for primary vasculitis (Used with permission. Watts R, Suppiah R, Merkel P, Luqmani R. Systemic vasculitis – is it time to reclassify? The way forward in approaching a diagnosis of vasculitis. *Rheumatology*, 2001;50:643–645)

disease can occur at any age, there is a skewing of the age distribution towards older age groups for both GPA and MPA with peak age of onset being 65–74 years. The majority of young patients with AAV have GPA and PR3-ANCA (Fig. 2). Older patients have worse renal function and there is also an increased proportion of these patients being diagnosed with crescentic glomerulonephritis on kidney biopsies (from 5 % in those <60 years to over 11 % in those >60 years of age). The relative frequencies of GPA and Churg-Strauss syndrome, but not MPA, are influenced by latitude with GPA and Churg-Strauss syndrome being more frequent in colder climates in both the North and South hemispheres. In addition, there are ethnic differences with GPA being less common in eastern Asian and black populations. Reports in Japan and China suggest that MPA is more common than GPA as was MPO-ANCA-associated disease compared to PR3-ANCA-associated disease.

Genetics

Genetic associations have been reported with polymorphisms of cytotoxic T lymphocyte associate antigen 4 (CTLA4), tyrosine-protein phosphatase non-receptor type 2, the third complement component, and the Fc gamma RIII immunoglobulin receptor. Familial segregation of GPA carries a relative risk of 1.6 for first degree relatives. Most associations have been with genes that encode proteins that are involved in the immune response such as the human leukocyte antigen (HLA) proteins, PTPN22m,

Vasculitis and the Kidney, Table 3 The chapel hill consensus conference definitions of the antineutrophil cytoplasmic antibody-associated vasculitides (Adapted from Jeanette (1994))

GPA (Wegener's granulomatosis)
Granulomatous inflammation involving the respiratory tract
Necrotizing vasculitis affecting small to medium-sized vessels (e.g., capillaries, venules, arterioles, and arteries)
Necrotizing glomerulonephritis is common
Microscopic polyangiitis
Necrotizing vasculitis, with few or no immune deposits, affecting small vessels (i.e., capillaries,venules, or arterioles)
Necrotizing arteritis involving small- and medium-sized arteries may be present
Necrotizing Glomerulonephritis is Very Common
Pulmonary capillaritis often occurs
Churg-Strauss syndrome
Eosinophil-rich and granulomatous inflammation involving the respiratory tract
Necrotizing vasculitis affecting small- to medium-sized vessels
Associated with asthma and eosinophilia

CTL4, and others. HLA-DPB1*0401 has been found to be a strong and reproducible risk factor for GPA but not MPA. In addition, some studies have suggested an association between AAV and the rare z allele of SERPINA which encodes for α-1 antitrypsin, a serine protease inhibitor that inhibits, among other targets, the enzymatic activity of PR3-ANCA. Deficient α-1 antitrypsin phenotypes develop more aggressive PR3-ANCA-associated vasculitis (Savage 2011).

Etiology, Pathogenesis, and Pathology

Environmental Associations

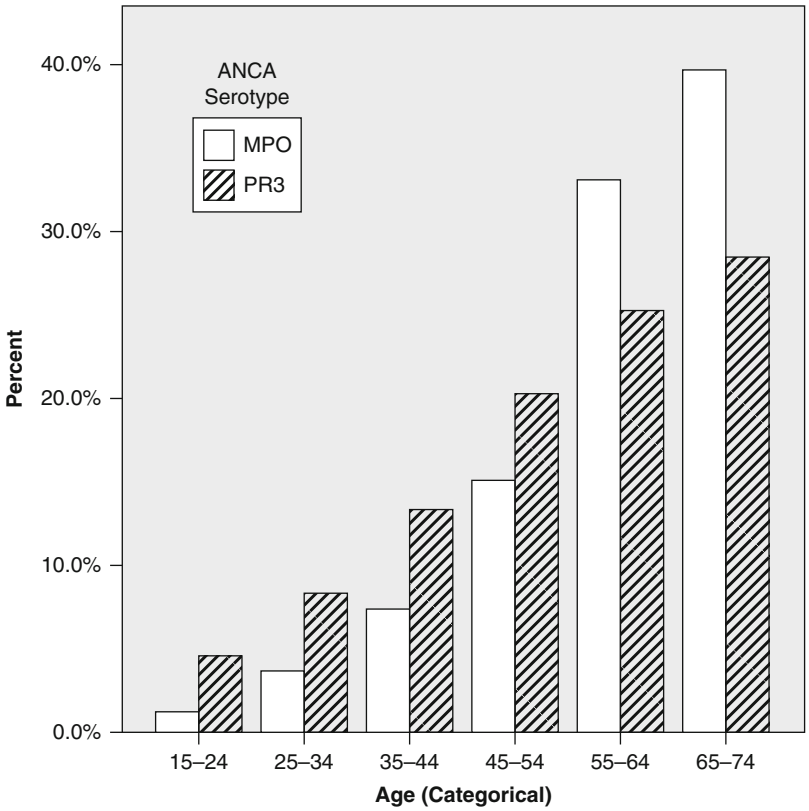
AAV has been associated with silica exposure and an increased incidence of MPO-ANCA vasculitis was reported in Kuwait in 1991 after the first Gulf war and after the Kobe earthquake in 1995. Whether other occupational exposures are etiologically related, such as heavy metals or insecticides, is unclear. Infections have also been linked to the pathogenesis of vasculitis (Hogan et al. 2007).

Pathogenic Role of ANCA

ANCA were identified in the 1980s as diffuse granular (cANCA) or perinuclear staining (pANCA) on indirect immunofluorescence (IIF). Proteinase 3 (PR3) and myeloperoxidase (MPO) antigens were subsequently identified as targets of cANCA and pANCA, respectively, and can be measured by ELISA. It should be noted however that 5–10 % of patients with small vessel vasculitis are ANCA negative. The pathogenic role of ANCA has been controversial because the pathology observed in AAV can occur without circulating ANCA, immune deposits are rarely present, and ANCA often persist without disease activity.

Neutrophils contain the target antigens for ANCA (PR3/MPO/LAMP) and are central in the development of vasculitis. Experimental studies have demonstrated that ANCA induce neutrophil activation, superoxide, and cytokine release and can cause neutrophil-mediated endothelial cytotoxicity. ANCA require neutrophil priming with tumor necrosis factor (TNF) which leads to translocation of proteinase 3 or myeloperoxidase from primary azurophilic granules in the cytoplasm to the cell membrane. In addition to binding to cell surface autoantigen, ligation of the Fc component of the ANCA antibody to neutrophil Fc receptors is necessary for intracellular signaling and cell activation. The interplay between chemokines, neutrophils, and ANCA IgG leads to the preferential recruitment of neutrophils to the microvascular sites (Hewins and Savage 2003).

Spontaneous and induced animal models (Xiao et al. 2002; Hamano et al. 2006) have demonstrated the ability of ANCA to cause a pauci-immune renal vasculitis in susceptible animal strains. Both immunocompetent and Recombinant Activating Gene (RAG-2) deficient mice that lack functioning T and B cells, receiving anti-myeloperoxidase antibodies, develop clinical features resembling those seen in human AAV, such as necrotizing vasculitis with crescentic glomerulonephritis. The evidence in humans is indirect, with drugs such as propylthiouracil causing drug induced AAV. Propylthiouracil



Vasculitis and the Kidney, Fig. 2 Age distribution of 735 AAV patients from 6 European Vasculitis Study Group (EUVAS) trials stratified according to ANCA subtype. The upper age limits of these trials are 75–80

accumulates within neutrophil granules and is thought to cause vasculitis possibly by increasing the immunogenicity of myeloperoxidase. Another possible example of the pathogenicity of ANCA includes case reports of a newborn child who developed glomerulonephritis and pulmonary hemorrhage after being born to a mother who had active MPO-ANCA vasculitis (Kallenberg 2011; Savage 2011).

The Innate and Adaptive Immune Response and the Infectious Link

The involvement of the respiratory tract in GPA has focused attention on the interaction between respiratory tract infection and a dysregulated immune response. Nasal colonization with *Staphylococcus aureus* is common (60–70 %) in patients with GPA and is associated with a higher relapse rate (relative risk of 7.2) (Kallenberg 2011). Treatment with sulfamethoxazole/trimethoprim reduces relapse rates by 60 %.

A possible role of *S. aureus* superantigens in stimulating the adaptive immune response has been suggested. The α-toxin from *S. aureus* is a potent activator of NLRP3 suggesting a potential link between infections and the proinflammatory effects of vasculitis. Bacterial strains expressing toxic shock staphylococcal toxin are implicated in relapsing disease (Stegeman et al. 1994). Damage to the respiratory tract resulting from vasculitic inflammation impairs its ability to eradicate microbial infection, and a cycle of vasculitis and recurrent infection develops. Cytokine-induced upregulation of endothelial adhesion molecules promotes leukocyte adhesion and injury and provides an additional mechanism by which inflammation secondary to infection can stimulate vasculitis.

Alternations within in the T cell populations include markedly reduced levels of T helper cells, skewing towards effector memory T cells, altered expression of co-stimulatory molecules, and

increased numbers of activated T cells. T cells in granulomatous lesions are overrepresented by a CD4+, CD28- subset which release gamma interferon and TNFα and have cytotoxic potential. A pathogenetic role for cytotoxic T cells has been shown in larger vessel arteritis and similar mechanisms are also likely to be important in smaller vessel disease (Weyand and Goronzy 2003). Circulating markers of T cell activation including the soluble interleukin 2 receptor are elevated. Deficient or dysfunctional Tregs and TH17 subsets have also been implicated. In experimental models, animals lacking IL-17A are protected against anti-MPO glomerulonephritis. Recent studies showing that a novel CD8+ T cell transcription signature can predict the likelihood of relapse in ANCA vasculitis suggest that changes in the T cell compartment can influence the course of disease (Fig. 3) (Abdulahad et al. 2009; Kallenberg 2011; Savage 2011).

The success of B cell depletion therapy with rituximab has highlighted a central role of B cells in pathogenesis. B cells which are present in granulomata and at sites of vasculitic injury have specificity for ANCA autoantigens and demonstrate features of affinity maturation.

Infections have also been implicated in the formation of the new type of ANCA directed against the human lysosomal associated protein (h-LAMP2). Kain et al. have suggested that anti-LAMP2 antibodies are important in the development of vasculitis with evidence of molecular mimicry between LAMP2 and the bacterial adhesion protein Fim-H found in gram-negative bacteria. Another infectious link is the production of anti-PR3 antibodies via an anti-idiotypic effect due to surface homology between the middle portion of complementary PR3 (cPR3) and proteins in *S. aureus* (Savage 2011).

Complement

Although AAV causes a "pauci-immune" glomerulonephritis, the complement system appears to be important in pathogenesis because C5 and Factor B knockout mice are protected against disease. C5a is an anaphylatoxin known to prime neutrophils for ANCA- induced respiratory burst, and mice that are deficient for the C5a receptor have been shown to be protected against glomerulonephritis (Savage 2011).

Pathology

AAV predominantly affects small blood vessels, capillaries, arterioles, and venules but may also affect muscular arteries and rarely larger arteries. Capillaritis in the glomerular tuft results in capillary thrombosis and infarction. This appears on biopsy as segmental fibrinoid necrosis and a secondary crescentic reaction within Bowman's capsule containing monocytes and epithelial cells. This progresses to involve the whole tuft and destroy the glomerulus. In addition, there is peri-glomerular and tubulointerstitial inflammation which may contain giant cells. There is considerable variety in the severity and proportion of fibrotic lesions between glomeruli. Obsolescent glomeruli and fibrotic crescents reflect previous vasculitic events and are associated with tubulointerstitial scarring. Capillaritis in pulmonary alveoli causes capillary rupture and hemorrhage into the alveolar space.

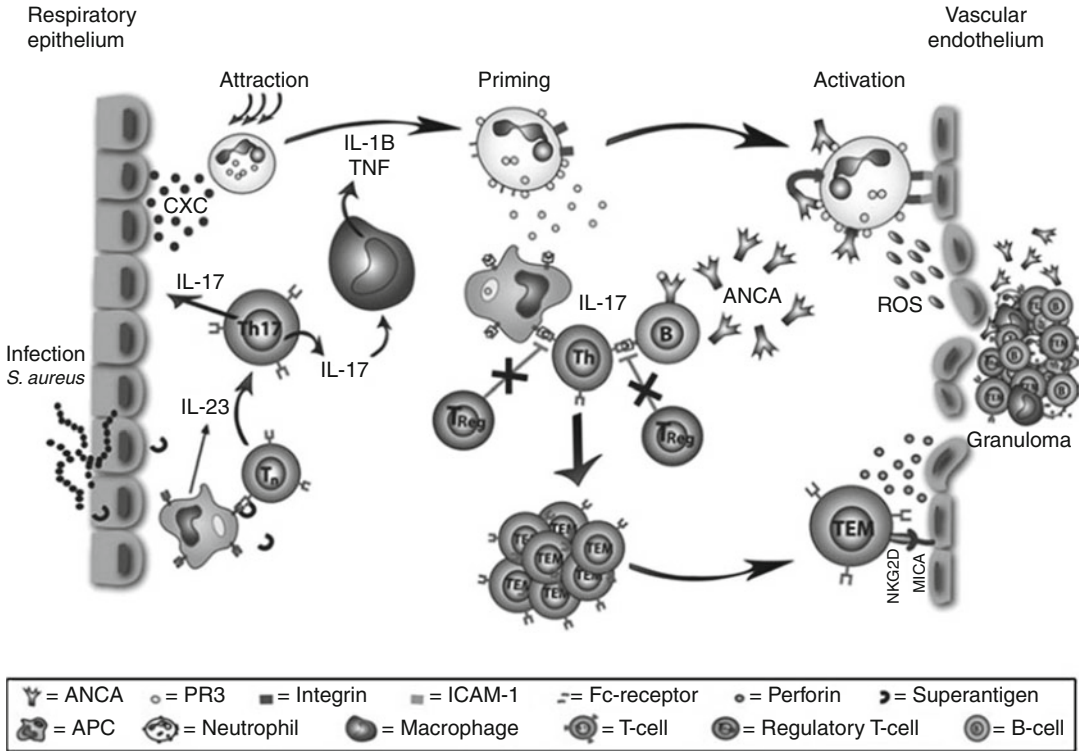
Approach to Diagnosis

Patients with primary systemic vasculitis vary in the pattern and severity of organ involvement, in their response to therapy, and in their subsequent disease course and prognosis. Diagnosis depends on the triad of clinical features, the serology and histology, and the exclusion of secondary causes. Appropriate treatment aims to obtain and sustain disease remission, but relapses are common, and refractory disease or chronic, persistent low-level disease activity represent therapeutic challenges (Hellmich et al. 2007).

Clinical Features

ANCA-Associated Vasculitis

Patients with ANCA-associated vasculitis typically have a prodromal phase of several months with constitutional symptoms such as fever, night sweats, polymyalgia, and weight loss. Half of all patients have chest and ear/nose/throat symptoms, and around 90 % have renal involvement. Other potentially affected organs include the



Vasculitis and the Kidney, Fig. 3 A proposed model representing innate and adaptive immune mechanisms that are supposedly involved in the pathogenesis of antineutrophil cytoplasmic autoantibody (ANCA)-associated systemic vasculitis (AASV). Superantigens and peptidoglycans from *Staphylococcus aureus* stimulate antigen-presenting cells (APC) in the respiratory tract to produce interleukin (IL)-23, which then induce proliferation of Th17 cells and release of IL-17. IL-17 acts further on both respiratory epithelium and tissue macrophages. In response to IL-17, bronchial epithelial cells secrete CXC chemokine that attract neutrophils to the infected tissue, whereas macrophages release proinflammatory cytokines, such as IL-1b and tumor necrosis factor (TNF)-a. These inflammatory cytokines cause priming of neutrophils (membrane expression of proteinase-3 (PR3)) and upregulation of adhesion molecules on their surface as well as on the vascular endothelium. Subsequently, primed neutrophils adhere to the endothelial cells. Released PR3 can be processed and presented by APC to Th cells. As regulatory T cells (TReg) fail to inhibit this autoimmune response in Wegener's granulomatosis, autoreactive T cells might undergo repeated stimulation by PR3-pulsed APC resulting in a pool of effectory memory T cells (TEM). Further, PR3-stimulated Th cells act on B cells and enhance the production of ANCA. Subsequently, ANCA activates neutrophils that adhere to endothelial cells, which results in local production of reactive oxygen species (ROS) and release of proteolytic enzymes that damage vascular endothelial cells. Moreover, the expanded population of CD4+ TEM cells, resulting from persistent activation of Th cells by PR3, upregulate their NKG2D protein and migrate to the peripheral blood and remain in the circulation during remission. When the disease becomes active, MICA protein will be upregulated on several vascular endothelial cells, especially in the kidney, which attract TEM cells to the inflammatory areas. The MICA protein on the target cells can bind to NKG2D on the TEM cells, which in turn enhances their cytotoxic function to kill the target cell in a perforin and granzyme-dependent way ending up in vasculitis (Used with permission from Abdulahad W, Stegeman C, Kallenberg C. Review article: The role of CD4+ T cells in ANCA-associated systemic vasculitis. Nephrology. 2009; 14: 26–32)

skin, eyes, lungs, and heart and the nervous system. Common presentations of AAV are summarized in Table 4.

Renal vasculitis is the most common cause of the syndrome of rapidly progressive glomerulonephritis (RPGN), accounting for 50–80 % of all cases. This syndrome consists of acutely deteriorating renal function and crescentic glomerulonephritis on kidney biopsy. Renal vasculitis is differentiated from other causes of

Vasculitis and the Kidney, Table 4 Common manifestations of ANCA-associated vasculitis (Adapted from Berden et al. (2012))

General	Malaise, myalgias, arthralgias, weight loss, headache, fevers, flu-like illness, headaches
Ear, nose, and throat	Increasing bloody-purulent nasal discharge with crusting (not responding to antibiotics)
	Slowly developing nasal stenosis with mid-facial pain
	Hearing loss
Eye	Unexplained conjunctivitis
	Uveitis, episcleritis
	Unilateral proptosis
	Paresis of the cranial nerves (especially oculomotor) nerves
Lungs	Cough and shortness of breath
	Possibly with bloody-purulent sputum (not responsive to antibiotics)
	Bilateral infiltrates on radiography (not responsive to antibiotics)
	Non-tuberculous cavitating lesions
	Alveolar hemorrhage
Skin	Bursts of small cutaneous vasculitis elements
	Purpura
	Ulcers
	Pyoderma gangrenosum
Kidneys	Hemproteinuria
	Worsening renal function
Nerves	Mononeuritis multiplex

glomerulonephritis by circulating immune reactants and renal immunofluorescence studies. For those with active renal vasculitis, urinary abnormalities will be presented and should therefore be sought in all patients with unexplained illness or where there is a suspicion of vasculitis. In these patients, hematuria and proteinuria is present, but may be confused with prostatic disease or urinary tract infection if the urine is not closely examined. The presence of red cell casts on urine microscopy reflects severe glomerular injury and is usually associated with crescentic glomerulonephritis.

Some 5 % of AAV patients present with simultaneous renal vasculitis and anti-glomerular basement membrane (GBM) disease (Levy et al. 2004). They are older, have more severe renal disease, and are more likely to have pulmonary involvement than other AAV cases. The serology demonstrates ANCA, usually MPO-ANCA positivity and anti-GBM antibodies. Renal histology reveals an aggressive crescentic glomerulonephritis, typically involving all glomeruli with linear IgG deposition on immunofluorescence.

Henoch-Schönlein Purpura

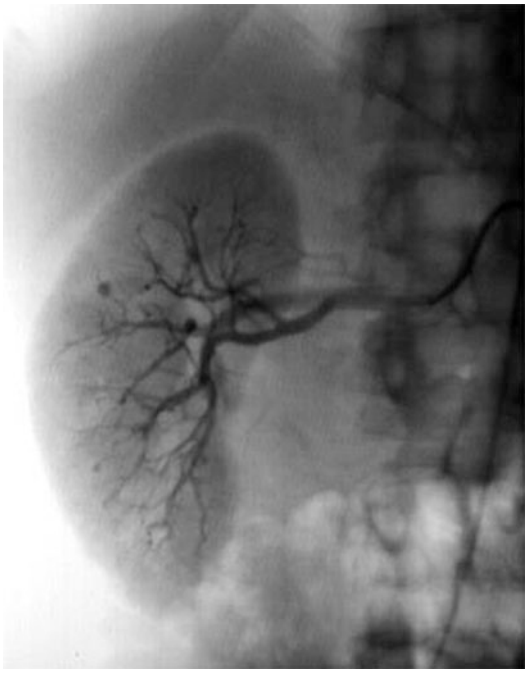
Henoch-Schönlein purpura occurs more frequently in children (15 cases/100,000 children/year) than adults (annual incidence 1/1,000,000). In adults it often pursues a relapsing course (Coppo et al. 1999). Nephritis occurs in 50–80 % in adults and 20–40 % in children. Patients can present with isolated hematuria, proteinuria, acute nephritis, or nephritic syndrome. By definition, extrarenal features of vasculitis including purpura, arthritis, and gastrointestinal involvement are present, but differentiation from other vasculitic syndromes requires the demonstration of IgA deposition on skin or renal biopsy. The renal presentation in Henoch-Schönlein purpura overlaps with that of IgA nephropathy but is more likely to pursue a rapidly progressive course and to have extra-capillary glomerular necrosis with crescents on biopsy.

Polyarteritis Nodosa and Takayasu Arteritis

Involvement of muscular arteries in the kidney in polyarteritis nodosa leads to regional infarction and hypertension (Fig. 4). The association of polyarteritis nodosa with microscopic vessel involvement, such as necrotizing glomerulonephritis, has been called "polyangiitis overlap syndrome" but is now classified as microscopic polyangiitis. A minority of patients with Takayasu arteritis have disease below the diaphragm (Class IV) which may involve the renal arteries causing renal artery stenosis, hypertension, reduced renal size, and renal impairment (Fig. 5).

Investigations

Investigations for AAV aim to rule out secondary causes of vasculitis and diseases that mimic vasculitis. More specific tests are done to confirm the diagnosis and determine the extent of



Vasculitis and the Kidney, Fig. 4 Arteriogram of a patient with polyarteritis nodosa showing numerous micro-aneurysms of renal vessels

involvement. This would usually involve serology, biopsy of relevant sites, and imaging studies (Table 5). Referral to a nephrologist, otolaryngologist, pulmonologist, ophthalmologist, or neurologist may be necessary to determine involvement of specific organs.

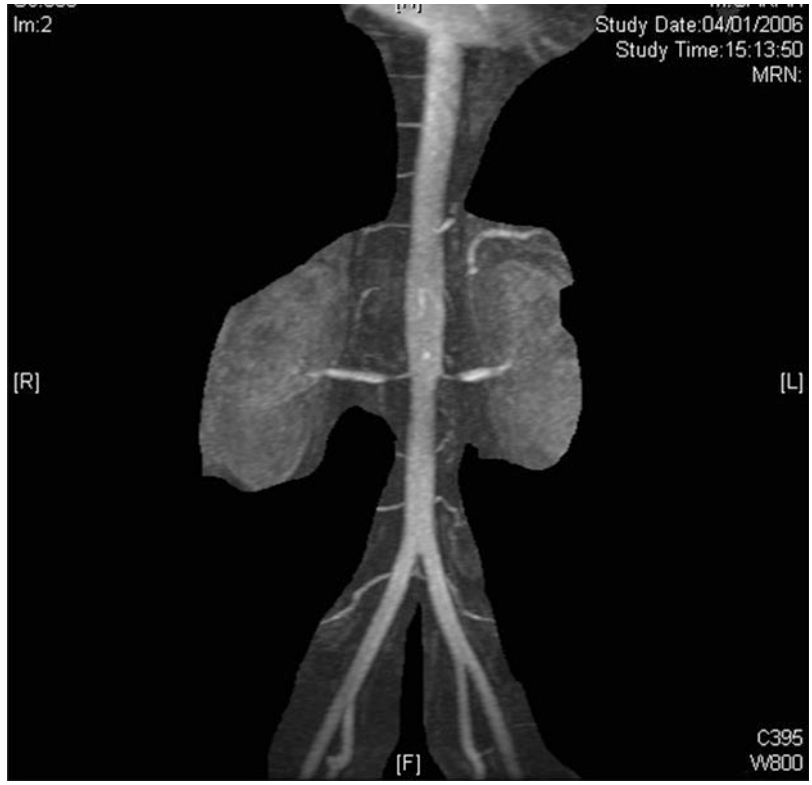
Serology

ANCA is now tested using a combination of IIF and ELISA. An international multicenter observational study found that IIF is more sensitive, but ELISA is more specific, and so IIF is usually used for screening and ELISA is used to confirm positive IIF results. ANCA positivity confirmed by a positive PR3-ANCA or MPO-ANCA has a predictive value above 95 % for the diagnosis of AAV with renal involvement in a patient with suspected nephritis. A minority (5–10 %) of patients with a pauci-immune, necrotizing, crescentic glomerulonephritis are ANCA negative, and ANCA is negative in other vasculitis syndromes, such as Henoch-Schönlein purpura or large vessel vasculitis. ANCA positivity by IIF (i.e., C-ANCA or P-ANCA) with negative PR3-ANCA and MPO-ANCA is still compatible with a diagnosis of AAV, but other chronic inflammatory processes that can produce a "false-positive" C-ANCA or P-ANCA need to be considered such as sinusitis or ulcerative colitis.

Although ANCA testing is widely used for the diagnosis of vasculitis, there is often confusion concerning the value of a negative result and the interpretation of positive results. A negative result has little value. In part, this is influenced by variable assay performance and differing referral practices. The role of ANCA in monitoring is more controversial with the best evidence suggesting that ANCA positivity during the remission phase indicates a higher risk of subsequent relapse.

Kidney Biopsy

Renal histology enables a more secure diagnosis to be made and is recommended for all patients with potential renal vasculitis. In the presence of ANCA positivity (both C-ANCA or P-ANCA and PR3-ANCA or MPO-ANCA), over 95 % of biopsies will show renal vasculitis, so it has been argued that biopsy is unnecessary. However, biopsy also permits diagnosis of concurrent conditions, such as anti-GBM disease or IgA nephropathy, and carries prognostic significance. Biopsy is strongly recommended when PR3-ANCA or MPO-ANCA is negative. The prognostic value of renal biopsy for ESRD may be helpful but is not sufficiently well determined to influence treatment in a particular individual. Microscopic hematuria persists for many months after the commencement of therapy for renal vasculitis despite normalization of the inflammatory markers CRP and ESR. It is unclear how well hematuria correlates with histological activity and what the criteria for repeat biopsy should be. The typical renal biopsy features in ANCA-associated vasculitis are a pauci-immune necrotizing glomerulonephritis with crescent formation. The histological severity of glomerular lesions is classified into focal, crescentic, fibrotic, and mixed groups with the risk of ESRD being lowest for focal and highest for fibrotic disease subgroups.



Vasculitis and the Kidney, Fig. 5 Takayasu arteritis with renal artery involvement (type IV). The angiographic classification of Takayasu arteritis involves 5 stages: type I: branches of the aortic arch; type IIa: ascending aorta, aortic arch, and branches of the aortic arch; type IIb: ascending aorta, aortic arch, and its branches and thoracic descending aorta; type III: thoracic descending aorta, abdominal aorta, and/or renal arteries; type IV: abdominal aorta and/or renal arteries; type V: features of types IIb and IV

Differential Diagnosis

Secondary causes of vasculitis and diseases mimicking vasculitis need to be excluded before a diagnosis of primary systemic vasculitis can be made (Table 2). Chronic inflammatory disorders such as bacterial endocarditis or rheumatoid arthritis can mimic vasculitis or induce a systemic vasculitis syndrome, such as an ANCA-associated vasculitis. Chronic bacterial infection may be obvious, as in cystic fibrosis or bronchiectasis, but occult endocarditis or abdominal sepsis should be considered. Tuberculosis and other non-vasculitic causes of pulmonary cavities can mimic GPA.

For those presenting with deteriorating renal function, other causes of rapidly progressive glomerulonephritis and acute renal failure need to be considered. Radiological assessment of renal size is typically normal in vasculitis; reduced renal size points to more chronic causes of renal disease. The presence of microscopic hematuria and proteinuria is nonspecific, occurring in other forms of renal and lower urinary tract inflammation and infection. Nephrotic range proteinuria may be found during the recovery phase of ANCA-associated vasculitis and in vasculitis associated with immune complexes, such as in cryoglobulinemia or Henoch-Schönlein purpura.

Treatment

AAV patients with renal vasculitis will usually progress to end-stage renal disease unless treated promptly. The progression of renal disease in rarer vasculitides, such as in Henoch-Schönlein purpura, has been less well studied and the role of therapy less established. The induction phase, of 3–6 months, aims to control active features of vasculitis. This is followed by the maintenance or remission phase, which could last 2–4 years, during which treatment is continued. Treatment is then slowly withdrawn but indefinite follow-up is required for the early detection of late relapse and the management of irreversible damage caused by the disease and its treatment. A major problem

Vasculitis and the Kidney, Table 5 Investigations for suspected primary systemic vasculitis

To exclude other causes (including secondary causes of vasculitis)	
Antinuclear antibody (ANA)	Antiphospholipid antibodies
Anti-GBM antibodies	
Complement levels	Serum electrophoresis
Consider infections	Viral (hepatitis B/C/HIV)
	Chronic Bacterial, e.g., endocarditis (ECHO) and
	blood cultures
Further confirmatory tests	
ANCA (MPO/PR3)	Cryoglobulins
Urine Dipstick and Microscopy	Renal biopsy (histology/ immunofluorescence/EM)
Renal ultrasound	Chest X-ray
Nerve conduction studies or electromyography	Angiogram
Biopsies of other sites: ENT, nerve, skin, lung	CT (chest/sinuses/head)
Peripheral eosinophilia	MRI
Bronchoscopy	
Referral to other specialists	
Nephrology	Renal biopsy
Ophthalmologist	? Episcleritis/uveitis
Ear, nose, and throat surgeon	? Assessment of ear, nose, and throat Involvement
	For example, granulomas, inflammation
	Hearing tests
Pulmonologist	Bronchoscopy

with current treatment is treatment toxicity that range from the risk of infections to hematological and urothelial malignancies.

Induction Therapy

Cyclophosphamide was introduced in the 1960s, and the combination of cyclophosphamide and corticosteroids now remains the "standard of care" for renal vasculitis. In the induction phase, cyclophosphamide is continued for 3–6 months, by which time vasculitis will have been controlled in 90 % of patients. Close monitoring of the full blood count is required for the early detection of cytopenias and appropriate dose adjustment. Prednisolone is commenced at high dose and tapered.

Additional treatment with high-dose intravenous methylprednisolone, 1,000–3,000 mg, is widely used for renal vasculitis without a firm evidence base and may be commenced on suspicion of the diagnosis before ANCA testing or renal histology is available. Plasma exchange improves the chances of renal recovery in those presenting in renal failure, with a serum creatinine >500 μmol/L (Jayne et al. 2007), but it is uncertain whether it also has a role in renal vasculitis with deteriorating renal function below this level or in severe non-renal presentations, such as diffuse alveolar hemorrhage.

Treatment intolerance or severe infections are the most common causes of treatment failure in the induction phase. Progressive disease is treated first with intravenous methylprednisolone and/or plasma exchange. If this fails or there is treatment intolerance or active disease persists beyond 6 months, then agents used for refractory vasculitis are employed (see below).

Rituximab has been directly compared to cyclophosphamide for remission induction in two randomized trials (Jones et al. 2010; Stone et al. 2010). They each demonstrated non-inferiority of rituximab as compared to cyclophosphamide in terms of efficacy, but neither trial demonstrated an early safety benefit of reducing or avoiding cyclophosphamide exposure.

Maintenance Therapy

The goals of maintenance therapy are to prevent disease relapse with less risk of drug toxicity then during the induction phase. Cyclophosphamide is withdrawn and replaced by azathioprine. Methotrexate is an alternative if serum creatinine is less than 1.8 mg/dl (Jayne et al. 2003; De Groot et al. 2005). Prednisolone is either continued in conjunction with an immunosuppressive or is withdrawn during the maintenance phase.

Thromboprophylaxis

Thromboembolic events, including pulmonary emboli, myocardial infarction, and stroke occur in 7–15 % of patients during the first year. Thromboprophylaxis may have an important role, although this has not yet been proven.

The Treatment of Refractory Vasculitis

Refractory vasculitis occurs rarely during the induction phase, but is more common later in the disease course, and is manifested by multiple relapses or a state of persistent disease activity. The re-administration of conventional agents including intravenous methylprednisolone, plasma exchange, and cyclophosphamide can control most acute cases of refractory disease. However, their use may be complicated by intolerance, intercurrent infection, or concern over cumulative cyclophosphamide exposure.

Other approaches to refractory disease include intravenous immunoglobulin (IVIg), B cell depletion, and T cell depletion. IVIg reduces levels of vasculitic activity in persisting or relapsing, reduces ANCA production, and is a useful adjunct short term, permitting reduction in immunosuppressive or steroid dosing. This is desirable in the face of active infection, in patients at high risk of infection, and in pregnancy. B cell depletion with rituximab has been shown to be more effective than further cyclophosphamide for relapsing or refractory disease and may be the preferred agent in this setting. T cell depletion with Alemtuzumab (CAMPATH 1-H), a humanized monoclonal anti-CD 52 that selectively depletes lymphocytes, has been shown to induce remissions in difficult to treat AAV, although relapses and adverse events are common.

Adverse Events of Therapy

The major early risk of combined cyclophosphamide and prednisolone treatment is sepsis, often associated with leukopenia which increases the septic risk more than fourfold. Older age and uremia greatly increase the risk of sepsis. A causal sequence of cyclophosphamide-induced neutropenia, sepsis and death, has been established, and cyclophosphamide dosing should aim to minimize neutropenia. Other side effects include hemorrhagic cystitis and infertility. Late risk includes urothelial and hematological malignancies. Steroid-related side effects are very frequent and include fluid retention, weight gain, hypertension, diabetes, and steroid-induced bone disease.

Prognosis and Outcomes

A recent study looking at the long-term outcome of AAV reported 1, 2, and 5 year survival rates of 88 %, 85 %, and 78 %, respectively, with a mortality ratio of 2.6 when compared to age- and sex-matched controls (Flossmann et al. 2011). The most common causes of early death were infection and active vasculitis; later deaths were caused by malignancy and cardiovascular events.

Creatinine at presentation remains the strongest predictor of both patient and kidney survival for renal vasculitis. Those presenting in renal failure have a particularly poor outcome, and earlier diagnosis is likely to improve outcome more than improved therapies.

For those presenting with renal impairment who respond to therapy, there is a gradual improvement in renal function over the first year. GFR may then remain stable for many years even if recovery is to a GFR below 30 ml/min. In this setting, vasculitis relapse with renal involvement carries a high risk of ESRD.

Cardiovascular events are common during periods of active vasculitis and may be caused by the increased pro-thrombotic tendency. There is a threefold increased risk of cardiovascular events in ANCA-associated vasculitis, which has been attributed to direct and indirect effects of vasculitis on blood vessels, chronic inflammation, and the consequences of chronic renal failure and medications.

Malignancy risk is increased approximately twofold. A wide distribution of malignancies is seen, with bladder malignancies overrepresented reflecting cyclophosphamide exposure. Rates of urothelial malignancy have fallen considerably over the last decade due to lower cyclophosphamide exposures. The extent to which pulsed intravenous cyclophosphamide regimens increase the risk of bladder malignancy is not known (de Groot et al. 2001).

The risk of relapse declines with time but follow-up should remain lifelong because late relapse can still occur with potentially devastating consequences. Treatment withdrawal is the strongest relapse predictor; other predictors are a diagnosis of GPA, PR3-ANCA, cardiovascular

disease due to vasculitis, persisting ANCA positivity, a previous history of relapse, or nasal colonization with *Staphylococcus aureus* in GPA. Worse renal disease, especially with a creatinine >200 μmol/L, is a predictor of a lower relapse risk (Walsh et al. 2012). ANCA levels are not closely related to disease activity, but the persistence of ANCA at 6 months after induction therapy or a rising ANCA level indicate relapse is more likely.

End-Stage Renal Disease and Transplantation

In renal-limited vasculitis, treatment with immunosuppression and prednisolone can be withdrawn once ESRD is reached. However, continued therapy may be required to control extrarenal vasculitic disease. Relapse rates of AAV are lower in patients with ESRD, but relapse, especially of the respiratory tract, may still occur. ESRD vasculitis patients have a higher incidence of infection which complicates therapy. The success of renal transplantation in AAV is similar to that for other nondiabetic causes of ESRD (Schmitt and van der Woude 2003). Transplantation reduces the risk of vasculitic relapse and can proceed in the face of a persistently positive ANCA.

Management of Other Vasculitic Syndromes Involving the Kidney

Henoch-Schönlein purpura: although nephritis is not prevented by prednisolone, this is commonly used to treat active renal disease, often in combination with an immunosuppressive. Plasma exchange has the rationale of removing IgA and IgA containing immune complexes and may be considered when there is deteriorating renal function.

Cryoglobulinemic vasculitis: when associated with hepatitis C, therapy is directed at controlling viral replication. Prednisolone may be required for initial therapy of inflammatory manifestations such as nephritis. Hepatitis C-negative, "essential," cryoglobulinemia is treated with glucocorticoids, with or without an immunosuppressive, and plasma exchange. Rituximab has led to remissions in refractory hepatitis C-associated and essential cryoglobulinemia.

Takayasu arteritis: prednisolone and an immunosuppressive are used to arrest progression of vascular disease, but renal artery involvement requires specific therapy if there is evidence of functional decline in the affected kidney. The stenoses are less amenable to angioplasty and stenting than in atheromatous renovascular disease, but this option may still be effective. Renal autotransplantation appears to be a useful alternative.

Controversies in Treatment of Vasculitis and Future Goals

The first consensus treatment guidelines were published in 2007 and highlight areas where evidence is lacking and no clear direction can be given. The duration of cyclophosphamide induction therapy has been reduced to less than 6 months, but it is not known whether much shorter courses followed by an alternative immunosuppressive will be as effective. Similarly, corticosteroid dosing has not been formally tested and there is a trend for doses to be reduced more rapidly. The duration of maintenance immunosuppression and corticosteroid therapy varies widely between 6 months and over 4 years. The short-term benefit of plasma exchange on renal recovery has been demonstrated, but it is not known whether this expensive intervention influences long-term mortality or ESRD rates; there is also controversy over its role in other severe vasculitis presentations, such as rapidly progressive glomerulonephritis without advanced renal failure, and in lung hemorrhage with respiratory failure.

Of the newer drugs under evaluation, rituximab is emerging as both safe and effective for obtaining sustained disease remission and may replace current regimens with long-term immunosuppression and corticosteroids. If rituximab use becomes widespread, this will reduce cyclophosphamide-related toxicity, but

it is unclear whether glucocorticoid exposure will also be reduced and what the implications of rituximab are on maintenance regimens or long-term outcomes.

Cross-References

- Giant Cell Arteritis
- Immune System and Kidney
- Indications for Biopsy in Autoimmune GN
- Polyarteritis Nodosa
- Vasculitis: Granulomatosis with Polyangiitis (Wegener's)
- Vasculitis: Henoch-Schönlein Purpura

References

Abdulahad WH, Stegeman CA, et al. Review article: the role of CD4(+) T cells in ANCA-associated systemic vasculitis. Nephrology (Carlton). 2009;14(1):26–32.

Berden A, Arda, G, Jagre D et al. Diagnosis and the management of ANCA associated vasculitis. BMJ, 2012;344:e26.

Coppo R, Amore A, et al. Clinical features of Henoch-Schonlein purpura. Italian Group of Renal Immunopathology. Ann Med Interne (Paris). 1999;150(2): 143–50.

de Groot K, Adu D, et al. The value of pulse cyclophosphamide in ANCA-associated vasculitis: meta-analysis and critical review. Nephrol Dial Transplant. 2001;16(10):2018–27.

De Groot K, Rasmussen N, et al. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2005;52(8):2461–9.

Flossmann O, Berden A, et al. Long-term patient survival in ANCA-associated vasculitis. Ann Rheum Dis. 2011;70:488–94.

Hamano Y, Tsukamoto K, et al. Genetic dissection of vasculitis, myeloperoxidase-specific antineutrophil cytoplasmic autoantibody production, and related traits in spontaneous crescentic glomerulonephritis-forming/Kinjoh mice. J Immunol. 2006;176(6): 3662–73.

Hellmich B, Flossmann O, et al. EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: focus on anti-neutrophil cytoplasm antibody-associated vasculitis. Ann Rheum Dis. 2007;66(5):605–17. Epub 2006 Dec 2014.

Hewins P, Savage CO. ANCA and neutrophil biology. Kidney Blood Press Res. 2003;26(4):221–5.

Hogan SL, Cooper GS, et al. Association of silica exposure with anti-neutrophil cytoplasmic autoantibody small-vessel vasculitis: a population-based, case-control study. Clin J Am Soc Nephrol. 2007;2(2):290–9. Epub 2007 Feb 2007.

Jayne D, Rasmussen N, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med. 2003;349(1):36–44.

Jayne DR, Gaskin G, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. J Am Soc Nephrol. 2007;20:20.

Jennette JC. Nomenclature and classification of vasculitis: lessons learned from granulomatosis with polyangiitis (Wegener's granulomatosis). Clin Exp Immunol. 2011;164(Suppl):7–10.

Jennette JC, Falk RJ, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum. 1994;37(2):187–92.

Jones RB, Cohen Tervaert JW, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. N Engl J Med. 2010;363:211–20.

Kallenberg CGM. Pathogenesis of ANCA-associated vasculitis, an update. Clin Rev Allergy Immunol. 2011;41(2):224–31.

Lane SE, Watts R, et al. Epidemiology of systemic vasculitis. Curr Rheumatol Rep. 2005;7(4):270–5.

Levy JB, Hammad T, et al. Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. Kidney Int. 2004;66(4):1535–40.

Savage COS. Pathogenesis of anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis. Clin Exp Immunol. 2011;164(Suppl):23–6.

Savige J, Gillis D, et al. International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). Am J Clin Pathol. 1999;111(4): 507–13.

Schmitt WH, van der Woude FJ. Organ transplantation in the vasculitides. Curr Opin Rheumatol. 2003;15(1): 22–8.

Stegeman CA, Tervaert JW, et al. Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. Ann Intern Med. 1994;120(1):12–7.

Stone JH, Merkel PA, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med. 2010;363:221–32.

Walsh M, Floßmann O et al. Risk factors for relapse of ANCA associated vasculitis. Arthritis Rheum. 2012;64:S42–S48.

Watts RA, Suppiah R, et al. Systemic vasculitis–is it time to reclassify? Rheumatology (Oxford). 2011;50(4): 643–5.

Weyand CM, Goronzy JJ. Giant-cell arteritis and polymyalgia rheumatica. Ann Intern Med. 2003;139(6):505–15.

Xiao H, Heeringa P, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest. 2002;110(7):955–63.

Vasculitis: Behçet

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Synonyms

"Silk Road" disease; Adamantiades-behçet disease

Definition

During the 1930 annual meeting of the Medical Association of Athens, the Greek ophthalmologist Benediktos Adamantiades presented a 20-year-old male patient with relapsing iritis, genital ulcers, and arthritis. Seven years later the Turkish dermatologist Hulusi Behçet described three patients with the tri-symptom complex of oral and genital ulcerations and hypopyon uveitis (Zouboulis and Keitel 2002). This condition is currently considered as a distinct, chronic, relapsing multisystem inflammatory disorder which affects the small and large vessels of the venous and arterial systems and is characterized by mucocutaneous, ocular, arthritic, vascular, and central nervous system manifestations.

Epidemiology

Behçet disease exists worldwide, but it is more prevalent in Mediterranean, Middle Eastern, and Far Eastern countries across the ancient trading route known as the "Silk Road." The highest rate (4 in 1,000 adults) is found in Turkey, followed by Israel, Northern China, Iran, Korea, Japan, Saudi Arabia, Iraq, Morocco, and Egypt. However, recent findings suggest that in Western Europe it is a more common disease than previously thought, perhaps as a result of underdiagnosis, exceeding even the prevalence of polyarteritis nodosa (Yazici et al. 2008).

Pathogenesis and Genetic Factors

Behçet disease can be viewed as a condition linking autoinflammation and autoimmunity (McGonagle and McDermott 2006). Since its etiology and pathogenic mechanisms remain essentially unknown (Mendoza-Pinto et al. 2010), some authors prefer to designate it as a syndrome instead of a disease. No specific immune cell type can be recognized in vasculitic lesions, immune complex deposition is rarely seen, and although both innate and adaptive immune responses are augmented, autoantibodies are usually absent. In addition to the distinct epidemiological features, a genetic contribution to the disease is supported by the high sibling recurrence risk ratio and the strong association with HLA-B51. However, this HLA association accounts for less than 20 % of the genetic risk (Gul and Ohno 2012). In two large genome-wide association studies, the HLA-B51 association was confirmed and a second, independent association within the MHC Class I region was found. Moreover, interleukin-10, interleukin-23 receptor, and interleukin 12 receptor β2 were identified as susceptibility loci, whereas the disease-associated interleukin-10 variant (rs1518111 A allele) was associated with diminished mRNA expression and low protein production (Mizuki et al. 2010; Remmers et al. 2010). Given the anti-inflammatory effects of interleukin-10, these findings support a role for this cytokine in the uncontrolled activation of innate and/or adaptive immune responses seen in these patients. An important role of TNF-mediated interactions in the perpetuation of inflammation is also supported by the impressive clinical responses after therapy with anti-TNF antibodies in many patients (Sfikakis et al. 2007; Arida et al. 2011).

Clinical Spectrum

As shown in the Table 1, there is a wide range of clinical manifestations in Behçet disease. For a number of these features, there is significant difference in prevalence between different ethnic

Vasculitis: Behçet, Table 1 Frequency of clinical manifestations of Behçet disease

Oral ulcers	97–99 %
Genital ulcers	85 %
Papulopustular lesions	55 %
Erythema nodosum	45 %
Uveitis	35–70 %
Arthritis	35–60 %
Superficial thrombophlebitis	25 %
Deep vein thrombosis	5–10 %
Aneurysms	3–8 %
Central nervous system involvement	10–25 %
Epididymitis	5–15 %
Gastrointestinal involvement	5–25 %

groups (Yazici et al. 2007). Recurrent oral ulcers, the cardinal disease feature, are usually painful and appear in the tongue, pharynx, buccal, and labial mucosal membranes, either as single or multiple lesions, which heal within 1 week without scarring. Genital ulcers resemble oral ulcers but are usually larger and deeper, and appear on the scrotum, less frequently on the penis, or on the vulva and vagina in women.

Skin lesions most often include erythema nodosum-like lesions, as well as pseudofolliculitis and acneiform nodules which can appear all over the body and they are not always hair follicle-associated.

Ocular involvement may be severe and any part of the eye can be affected. Anterior uveitis with or without hypopyon, vitritis, and relapsing inflammation of the posterior segment with vasculitis, retinitis, optic disc swelling, and cystoid macular edema may occur.

Joint involvement is usually a monoarthritis affecting the knee or the ankle or oligoarthritis. Erosive changes are rare. Superficial thrombophlebitis is transient and can be misdiagnosed as erythema nodosum.

Thrombosis may occur in many different sites including the chambers of the heart, veins (iliofemoral, superior or inferior vena cava, pulmonary, axillary, brachial, and hepatic), and dural sinus. Arterial aneurysms may develop at the abdominal aorta, iliac, femoral, popliteal, carotid, and renal arteries and can rupture suddenly. Central nervous system involvement includes parenchymal and non-parenchymal (cerebral venous thrombosis or arterial aneurysm) lesions.

Epididymitis is manifest by testicular pain and is mostly self-limited.

Gastrointestinal involvement is particularly frequent in patients from the Far East; single or multiple deep penetrating ulcers develop mostly in the terminal ileum, the ileocecal region, and the colon. These lesions may mimic Crohn's disease.

Diagnosis

The diagnosis of Behçet disease is entirely clinical and requires the exclusion of other diagnoses based on clinical presentation. A careful past medical history is mandatory due to the relapsing-remitting course of the disease. There is no pathognomonic or specific laboratory test and HLA-B51 testing is not recommended for diagnostic purposes. The differential diagnosis is wide and depends on the given constellation of clinical manifestations. For example, multiple sclerosis should be ruled out in patient with central nervous involvement and uveitis, Takayasu's arteritis in a patient with arterial lesions, or inflammatory bowel disease in a patient with intestinal involvement and arthritis.

According to the widely accepted international criteria (Criteria for diagnosis of Behcet's disease 1990), a patient can be diagnosed with Behçet disease (with a sensitivity of 85 % and specificity of 96 %) if, in the absence of other explanations, the patient has recurring oral ulcerations (aphthous or herpetiform) observed by the physician or the patient at least three times in a 12-month period, plus at least any two of the following:

(a) Recurrent genital aphthous ulceration or scarring
(b) Eye lesions: anterior uveitis, posterior uveitis, cells in the vitreous by slit lamp examination, or retinal vasculitis observed by an ophthalmologist
(c) Skin lesions: erythema nodosum, pseudofolliculitis, papulopustular lesions, or acneiform nodules in postadolescent patients not on corticosteroids

(d) A positive pathergy test (nonspecific skin hyper-reactivity in response to minor trauma) read by a physician at 24–48 h

Natural Course and Prognosis

Behçet disease is rarely seen in children or in the elderly. The typical onset occurs in the third or fourth decade of life, but recurrent oral ulceration, which is most often the first symptom, may begin many years before the diagnosis is established. A male preponderance in seen in Mediterranean and Middle Eastern countries, but women are more commonly affected in the Far East. Familial cases have been reported (Kural-Seyahi et al. 2003). A remitting and relapsing course is characteristic of the disease, which abates in the majority of patients after 20 years. However, morbidity and mortality are significant. Ocular involvement is the leading cause of morbidity and, if left untreated, may result in blindness in more than 70 % of those affected. Young males have the worst prognosis and major vessel and neurological involvement, both of which may occur even 10 years after diagnosis, are the main causes of death (Saadoun et al. 2010). HLA-51 status has no prognostic significance.

Management

Management must be individualized, balancing the risks of therapy with the putative efficacy of a given approach. The aim of treatment is to maintain quality of life, prevent irreversible damage (which usually occurs early in the course of disease, especially in the high-risk group of young men) and prevent exacerbations of urogenital, cutaneous, and joint manifestations.

For self-limited manifestations such as oral ulcers, genital ulcers, papulopustular lesions, erythema nodosum, arthritis, epididymitis, and superficial thrombophlebitis, the aim is to control symptoms. Collaboration between different specialties and the use of immunosuppressive agents are mandatory for serious organ involvement, such as ocular involvement, major vessel disease, central nervous system, and gastrointestinal involvement. Adequately powered, randomized, and controlled clinical trials are few.

Corticosteroids, colchicine, cyclosporin-A, interferon-a, and cyclophosphamide, alone or in combination, are used but none of these represents a cure. Among the various immunosuppressive drugs, azathioprine remains the most useful (Yazici et al. 1990). The introduction of anti-TNF monoclonal antibodies during the last decade represents a significant advancement in the management of patients with severe, refractory manifestations and especially in relapsing sight-threatening involvement of the posterior eye segment (Sfikakis et al. 2001; Arida et al. 2011). Thalidomide, antibiotics, antiviral agents, methotrexate, sulfasalazine, chlorambucil, FK506, mycophenolate mofetil, rituximab, anti-IL-1 therapy, and stem cell transplantation have been used with variable results.

Evidence-based recommendations for the management of Behçet disease, supplemented where necessary by expert opinion, have been developed by a multidisciplinary expert committee based on systematic literature search through December 2006 (Hatemi et al. 2008). The following recommendations related to the eye, skin, mucosal, and joint disease are largely evidence-based: Any patient with inflammatory eye disease affecting the posterior segment should be on a treatment regime, which includes azathioprine and systemic corticosteroids. If the patient has severe eye disease defined as more than 2 lines of drop in visual acuity on a 10/10 scale and/or retinal disease (retinal vasculitis or macular involvement), it is recommended that either cyclosporine A or infliximab be used in combination with azathioprine and corticosteroids; alternatively interferon-alpha with or without corticosteroids could be used. The decision to treat skin and mucosa involvement will depend on the perceived severity by the physician and the patient. Topical measures (i.e., local steroids) should be the first line of treatment for isolated oral and genital ulcers as well as for acne-like lesions. Colchicine should be preferred when the dominant lesion is genital ulceration or erythema nodosum. Azathioprine, interferon-α, and TNF

antagonists may be considered in resistant cases. In most patients arthritis can be managed with colchicine.

The following recommendations on vascular disease, neurological and gastrointestinal involvement are based largely on expert opinion and uncontrolled evidence from open trials and observational studies: For the management of acute deep vein thrombosis, corticosteroids, azathioprine, cyclophosphamide, or cyclosporine is recommended. For the management of both pulmonary and peripheral arterial aneurysms, cyclophosphamide and corticosteroids are recommended. Anticoagulants, antiplatelet or fibrinolytic agents are not recommended; these agents may result in fatal bleeding, in the event of a coexisting pulmonary arterial aneurysm. Anticoagulants, antiplatelet, or fibrinolytic agents may cause fatal bleeding if a coexisting pulmonary arterial aneurysm dissects or ruptures. Therefore, these agents should be avoided (Hatemi et al. 2008). However, some experts recommend anticoagulation for venous thrombotic events.

For the management of gastrointestinal involvement, agents such as sulfasalazine, corticosteroids, azathioprine, TNF antagonists, or thalidomide should be tried before surgery, except in emergencies. There are no controlled data to guide the management of central nervous involvement. Due to its potential neurotoxicity, cyclosporine A should not be used in Behçet disease patients with central nervous system involvement unless necessary for intraocular inflammation. For brain parenchymal involvement, corticosteroids, interferon-alpha, azathioprine, cyclophosphamide, methotrexate, and TNF antagonists may be effective. For dural sinus thrombosis corticosteroids are recommended. Dural sinus thrombosis should be treated with corticosteroids (Hatemi et al. 2008) although anticoagulation is also recommended by some experts.

Cross-References

- ▶ Autoinflammatory Diseases
- ▶ Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis

References

Arida A, Fragiadaki K, Giavri E, Sfikakis PP. Anti-TNF agents for behçet disease: analysis of published data on 369 patients. Semin Arthritis Rheum. 2011;41:61–70.

Criteria for diagnosis of Behcet's disease: international study group for Behcet's disease. Lancet. 1990;335:1078–80.

Gul A, Ohno S. HLA-B*51 and Behçet disease. Ocul Immunol Inflamm. 2012;20:37–43.

Hatemi G, Silman A, Bang D, Bodaghi B, Chamberlain AM, Gul A, Houman MH, Kotter I, Olivieri I, Salvarani C, Sfikakis PP, Siva A, Stanford MR, Stubiger N, Yurdakul S, Yazici H. EULAR recommendations for the management of behcet disease. Ann Rheum Dis. 2008;67:1656–62.

Kural-Seyahi E, Fresko I, Seyahi N, Ozyazgan Y, Mat C, Hamuryudan V, Yurdakul S, Yazici H. The long-term mortality and morbidity of behçet syndrome: a 2-decade outcome survey of 387 patients followed at a dedicated center. Medicine (Baltimore). 2003;82:60–76.

McGonagle D, McDermott MF. A proposed classification of the immunological diseases. PLoS Med. 2006;3(8):e297.

Mendoza-Pinto C, García-Carrasco M, Jiménez-Hernández M, Jiménez Hernández C, Riebeling-Navarro C, Nava Zavala A, Vera Recabarren M, Espinosa G, Jara Quezada J, Cervera R. Etiopathogenesis of Behcet's disease. Autoimmun Rev. 2010;9:241–5.

Mizuki N, Meguro A, Ota M, Ohno S, Shiota T, Kawagoe T, Ito N, Kera J, Okada E, Yatsu K, Song YW, Lee EB, Kitaichi N, Namba K, Horie Y, Takeno M, Sugita S, Mochizuki M, Bahram S, Ishigatsubo Y, Inoko H. Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behçet's disease susceptibility loci. Nat Genet. 2010;42:703–6.

Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, et al. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behçet's disease. Nat Genet. 2010;42:698–702.

Saadoun D, Wechsler B, Desseaux K, Le Thi huong D, amoura Z, resche-rigon M, cacoub P. Mortality in Behcet's disease. Arthritis Rheum. 2010;62:2806–12.

Sfikakis PP, Theodossiadis PG, Katsiari CG, Kaklamanis P, Markomichelakis NN. Effect of infliximab on sight-threatening panuveitis in Behçet's disease. Lancet. 2001;358:295–6.

Sfikakis PP, Markomichelakis N, Alpsoy E, Assaad-Khalil S, Bodaghi B, Gul A, et al. Anti-TNF therapy in the management of Behcet's disease: review and basis for recommendations. Rheumatology (Oxford). 2007;46:736–41.

Yazici H, Pazarli H, Barnes CG, Tuzun Y, Ozyazgan Y, Silman A, Serdaroglu S, Oguz V, Yurdakul S, Lovatt GE, et al. A controlled trial of azathioprine in Behcet's syndrome. N Engl J Med. 1990;322:281–5.

Yazici H, Fresko I, Yurdakul S. Behcet's syndrome: disease manifestations, management, and advances in treatment. Nat Clin Pract Rheumatol. 2007;3:148–55.
Yazici H, Seyahi E, Yurdakul S. Behcet's syndrome is not so rare: why do we need to know? Arthritis Rheum. 2008;58:3640–3.
Zouboulis CC, Keitel W. A historical review of early descriptions of adamantiades- Behcet's disease. J Invest Dermatol. 2002;119:201–5.

Vasculitis: Granulomatosis with Polyangiitis (Wegener's)

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Synonyms

Granulomatosis with polyangiitis; Wegener's granulomatosis

Definition

Granulomatosis with polyangiitis (GPA) is a small and medium-sized vessel vasculitis that is associated with antineutrophil cytoplasmic antibodies (ANCA) in over 80 % of cases. Tissue injury involves both parenchyma and vessels. Vascular injury is not a feature of all lesions. While GPA commonly involves the upper respiratory tract, lungs, and kidneys, any organ system can be affected. Clinical course varies, depending on distribution of organs affected and rate of progression. If not treated or inadequately treated, GPA is usually life-threatening.

Genetic Background

Strong genetic associations with GPA have not been found, and familial cases are rare. Nonetheless, the relative risk for GPA in 1st degree relatives is 1.56 (Knight et al. 2008). Increasing numbers of genetic polymorphisms have been found to be associated with GPA, many of which are integral to the immune system. The strongest HLA associations have been seen with HLA DPB1*0401 and even stronger with the extended haplotype HLA DPB1*0401/RXRB03 (Table 1) which regulates apoptosis and is located in the MHC class II region (Jagiello et al. 2004). Several other gene alleles related to apoptosis have been reported to be associated with GPA although less strongly than with HLA DPB1*0401/RXRB03. This suggests that alterations in regulation of apoptosis in antigen-presenting cells may play a role in the etiology of GPA.

PTPN22 affects the signalling threshold of the T-cell receptor, binding intracellular tyrosine kinases and regulating phosphorylation of key kinases. The PTPN22 620W allele has been associated with T-cell activation and has been associated with a number of autoimmune diseases including type 1 diabetes, seropositive rheumatoid arthritis, and systemic lupus erythematosus. An increase in the PTPN22 620W allele has been seen in ANCA-positive GPA patients. Significant increases in this allele were not found in ANCA-negative patients compared with controls (Jagiello et al. 2005).

The neutrophil membrane expression pattern of proteinase 3 (PR3) is bimodal with either high or low expression patterns. This appears to be genetically determined as there is a stronger correlation between the expression patterns in monozygotic twins than dizygotic twins. Patients with ANCA-associated vasculitis have been found to have higher expression of PR3 on neutrophils. Overexpression of the G allele in the A-564G polymorphism of the PR3 gene leads to increased expression and is associated with GPA (Gencik et al. 2000). Since surface expression of PR3 on neutrophils is important to the formation of anti-PR3-ANCA complexes, this is a genetic link which could contribute to pathogenesis in a predisposed host.

Cytotoxic T-lymphocyte-associated protein4 (CTLA4) is found on $CD4^+$ T cells which controls interactions with B cells and dendritic

Vasculitis: Granulomatosis with Polyangiitis (Wegener's), Table 1 Selective list of genetic associations in GPA

Author	Gene	Effect	Population	Cases (control)	Odds ratio	P value
Jagiello et al.	DPB1*0401/RXRB03	↓ apoptosis of antigen presenting cells	German	150(100)	6.41	7.13×10^{-17}
Jagiello et al.	PTPN22 620W	↓ threshold for activation of T-cell receptor	German	199(399)	ANCA (+) 2.01	0.0002
					ANCA (−) 0.5	0.24
Gencik et al.	PRTN3-564G	↑ surface expression of PR3	German	67(129)	0.5	<0.01
Giscombe et al.	CTLA4-318T	↑ T-cell activation	Sweden	32(122)	3.26	<0.05
Borgmann et al.	α1-AT Z allele	α1-AT polymer formation induced inflammation	Germany	79(752)	3.8	<0.0001
Dijstelbloem et al.	FcyRIIa_RR131 + FcyRIIIa-FF158	Binding of lgG	Netherlands	91(154)	4.60	0.0092

Adapted from Willcocks et al. Arthritis Res Therapy. 2010;12:202

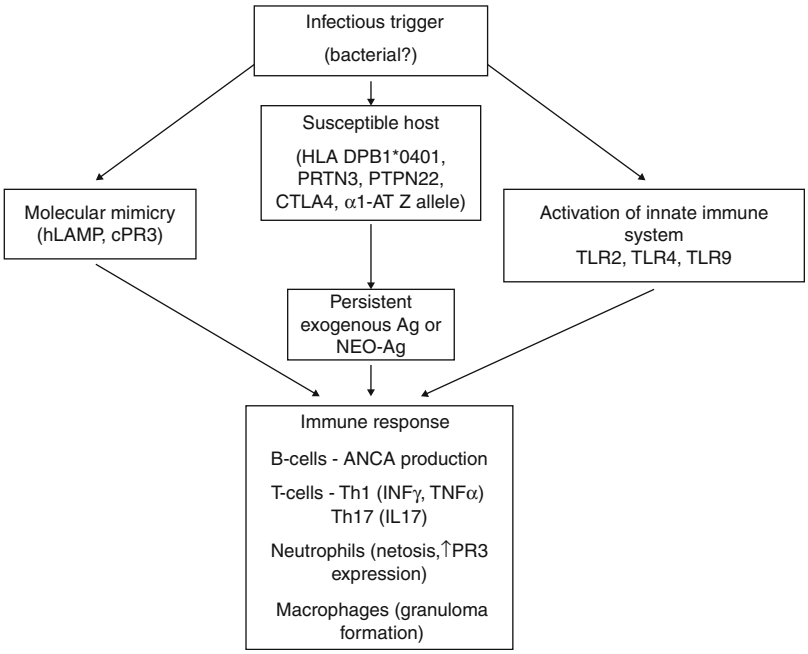
cells. As opposed to CD28 which enhances activity of T cells, CTLA4 is responsible for downregulating T-cell activation. A microsatellite polymorphism of the CTLA4 gene can result in longer alleles. Messenger ribonucleic acid (mRNA) produced from longer alleles is less stable and results in less CTLA4 production. The longer alleles are seen more frequently in GPA patients compared to healthy controls (53 % vs 30 %) (Zhou et al. 2004). In addition, there is a higher frequency of a single nucleotide polymorphism in the promoter region (CTLA4-318T) of CTLA4 in GPA patients (Giscombe et al. 2002). Reduction of inhibition of T-cell activation mediated by CTLA4 could produce an extreme T lymphocyte response.

Alpha 1-antitrypsin (α1-AT) serves an important role in neutralization of free PR3. The plasma level of α1-AT is controlled by codominant alleles. The M allele is the wild-type allele, while Z and S alleles have been associated with α1-AT deficiency. Furthermore, the Z allele has been associated with α1-AT polymer formation which accumulates within the endoplasmic reticulum. These polymers have known proinflammatory effects. In a recent large study from the United Kingdom, the Z allele was shown to have a higher association with GPA relative to healthy controls (HR = 2.54, CI 1.78–3.64, $p < 0.0001$). In patients with ANCA-associated vasculitis with the Z allele, there was a significantly reduced serum α1-AT concentration as well as evidence of polymer formation in the kidney of a patient with MZ phenotype with active crescentic glomerulonephritis. This is suggestive of a role for this allele in pathogenesis. However, the low frequency of the Z allele in GPA (4–8 %) argues against it playing a role in the majority of patients (Morris et al. 2011).

While genetic associations have been found that may contribute to dysregulation of the immune system, given the low prevalence of disease in first-degree relatives they each likely play a limited role. It remains plausible that the predisposition to GPA is related to polygenic factors that have not yet been identified (Fig. 1).

Environmental Factors

The high prevalence of systemic features and symptoms that mimic infection in the upper and lower airway has led many authors to suggest a role for an airborne trigger in GPA. An infectious etiology was suspected in the earliest descriptions of this disease by both Klinger (1931) and Wegener (1990). Early studies by Raynauld and colleagues suggested a higher incidence of new onset disease in spring and winter (Raynauld et al. 1993). However, data from the National Institutes of Health show no seasonal variation in regards to hospitalizations for GPA



Vasculitis: Granulomatosis with Polyangiitis (Wegener's), Fig. 1 Proposed model of pathogenesis

(Cotch et al. 1996). Clustering of cases within specific geographic regions is also suggestive of an environmental trigger(s) (Cotch et al. 1996).

Nasal carriage with *Staphylococcus aureus* has been shown to increase the risk of GPA relapse. Patients carrying a *S. aureus* strain that produces the super antigen toxic shock syndrome toxin-1 have the highest risk of relapse (Popa et al. 2007). In addition, treatment with trimethoprim-sulfamethoxazole (T/S) reduces the risk of relapse. It is tempting to attribute the effect of T/S to elimination of *S. aureus* in the upper airways; however, T/S may also have a primary anti-inflammatory effect through inhibiting formation of oxygen-derived radicals in activated neutrophils and/or immunosuppressive effects through antagonism of folic acid metabolism (Stegeman et al. 1996). Because T/S has been shown to reduce only upper airway symptoms, not pulmonary, renal, or other relapses, there is some question whether the benefit of T/S was actually on sino-nasal infection reduction and not disease relapse.

Infections have also been implicated in the formation of ANCA antibodies. Many infectious agents, including those associated with endocarditis, chronic infections, and parasites (Pudifin et al. 1994), have been variably associated with antibodies to either PR3 or MPO, and other lysosomal enzymes (Bonaci-Nikolic et al. 2010). Molecular mimicry is one proposed mechanism through which infections can induce ANCA. For instance, antibodies to lysosomal membrane protein-2 (LAMP-2) represent a newly described subtype of ANCA. Human LAMP-2 (hLAMP-2) antibodies, highly prevalent in pauci-immune focal necrotizing glomerulonephritis (FNGN), have been shown to activate neutrophils. hLAMP-2 may mediate damage to endothelium in vitro in concert with polymorphonuclear cells (PMNs) or independent of PMNs. It has also been shown to cause pauci-immune FNGN in rodents. hLAMP-2 has considerable homology to the adhesion molecule FimH found on fimbriated bacteria. Rats immunized with FimH develop pauci-immune FNGN and develop antibodies to hLAMP-2 (Kain et al. 2008). Further studies of serum from untreated patients with ANCA-associated vasculitis associated with pauci-immune FNGN from multiple cohorts have shown a high frequency of antibodies hLAMP-2 at presentation in three separate

assays (80–90 %). The antibodies become undetectable with treatment and are frequently detectable again in over half of patients during relapse (Kain et al. 2012). These finding suggest a role for anti-LAMP-2 antibodies in pathogenesis and provide a link between bacteria and disease. The observations need to be confirmed by larger cohorts.

Furthermore, *S. aureus* has homology with the middle portion of complementary PR3 (cPR3) and can induce cPR3 antibodies. cPR3 is elevated in up to one third of PR3-ANCA-positive patients and found in animal models to generate anti-PR3-ANCA through an anti-idiotype immune response (Hewins et al. 2011).

These examples demonstrate several potential mechanisms through which infections could trigger vasculitis through systemic activation of granulocytes, molecular mimicry between bacterial and host proteins, and induction of antibodies by bacterial super antigens (Fig. 1).

There has been less convincing evidence regarding noninfectious triggers. Some studies have implicated silica exposure as well as farming as risk factors. The majority of these studies have utilized questionnaires which are subject to recall bias. Other studies using population registries have reported no correlation between various occupations including those having high risk for silica inhalation (e.g., farming) and GPA (Lane et al. 2003; Knight et al. 2010). To date, there is not enough convincing evidence to implicate a particular noninfectious trigger in pathogenesis.

ANCA

ANCA is associated with GPA in about 80 % of cases. ANCA is also present in most patients with microscopic polyangiitis (MPA) and in about 38 % of cases of eosinophilia with granulomatosis and polyangiitis (EGPA or Churg-Strauss vasculitis). Because up to 20 % of GPA patients can be ANCA negative, questions have been raised about test sensitivity vs. the existence of a truly ANCA-negative GPA subset. If the latter were not a result of test inadequacy, one would have to conclude that ANCA is not essential to disease etiology but may still be important in pathogenesis (i.e., enhancing an established immuno-inflammatory process). ANCA targets proteins within the primary (azurophilic) and secondary granules of neutrophils, within lysosomes of monocytes, and in some cases, proteins that reside only in the cytoplasm. Those antigens most relevant to vasculitis are proteinase 3 (PR3), myeloperoxidase (MPO), and possibly hLAMP-2. While most patients with GPA are PR3-ANCA positive, up to 20 % of patients may have ANCA directed to MPO (and conversely for MPA, wherein 80 % of those positive are anti-MPO and up to 20 % are anti-PR3).

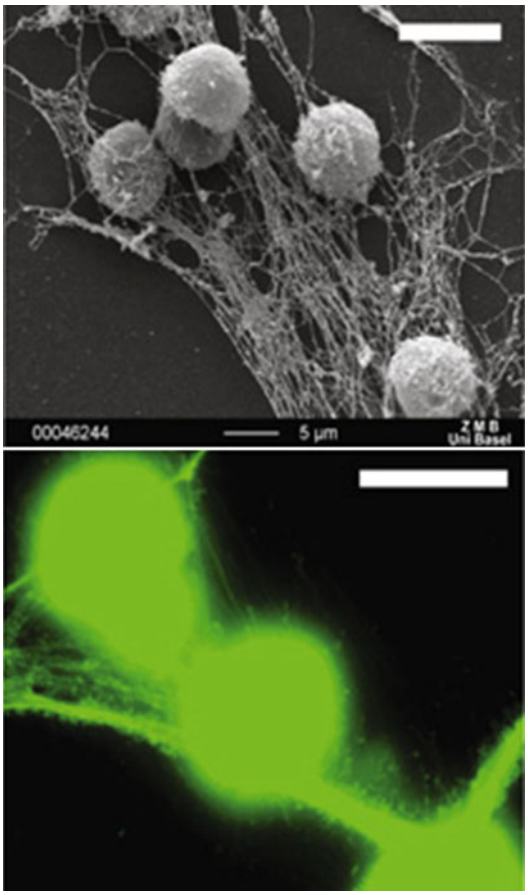
Experimental models have supported a role of PR3-ANCA antibodies in the pathogenesis of GPA. In vitro PR3-ANCA is able to activate neutrophils to produce oxygen species and release lytic enzymes. However, the neutrophils must be "primed" through tumor necrosis factor (TNF)-α, interleukin (IL)-1β, or complement factor C5a. TNF-α has several effects on neutrophils including upregulation of PR3 and other Ag-enzymes on the cell surface, making it available to PR3-ANCA. The expression of PR3 on neutrophils is variable among individuals and seems genetically determined. There are currently no good animal models for GPA to help directly link PR3-ANCA antibodies to pathogenesis. However, there are better animal models of small vessel vasculitis induced by MPO-ANCA. When splenocytes from MPO-deficient mice immunized with MPO are transferred to $Rag2^{-/-}$-immunodeficient mice, the recipients develop glomerulonephritis, granulomatous inflammation, and systemic necrotizing vasculitis. While this proof of concept model is compelling, patients with GPA are not $Rag2^{-/-}$ equivalents. Similar models with PR3-ANCA do not induce similar phenotypic changes (Kallenberg 2008).

Most of the PR3-ANCA in GPA patients with active disease has been shown to interfere with the enzymatic activity and with complex formation between PR3 and α1-antitrypsin, an inhibitor of PR3. Epitopes on PR3 targeted by ANCA include both those dependent on the 3D

structure of PR3 and those reactive to linear epitopes. A better understanding of ANCA-targeted epitopes is likely to improve our understanding of GPA disease expression (van der Geld et al. 2004).

NETosis (Neutrophil Extracellular Chromatin Traps, NETs)

Recently a newly recognized process that involves neutrophil cell death has shed light on the capture and killing of invading extracellular microbes. The process involves release of nuclear or mitochondrial chromatin from PMNs undergoing cell death, independent of apoptosis (Fig. 2). These structures also contain cytoplasmic and granular antimicrobial proteins. NETs are formed in response to a variety of proinflammatory stimuli (LPS, IL-8, and TNF) and microorganisms. In vitro, isolated neutrophils primed with TNF-α formed NETs more frequently when incubated with purified IgG from individuals with small vessel vasculitis compared to IgG from normal controls. In vivo evidence also supports the formation of NETs in kidney biopsies from patients with small vessel vasculitis with active kidney disease, and these NETs are coated with MPO and PR3. DNA NETs have been demonstrated to cause acute damage to small blood vessels during sepsis and may have a pathological role in small vessel vasculitis as well. Indeed, *S. aureus* is known to strongly induce NET formation (Kessenbrock et al. 2009). This observation lends additional support to the hypothesis that infection may be the initial trigger for disease.



Vasculitis: Granulomatosis with Polyangiitis (Wegener's), Fig. 2 *Top panel* – High resolution scanning electron microscopy of neutrophil NET's. *Bottom panel* – Syntox green staining showing high concentration of DNA in NET's (Gupta et al. 2010)

Toll-Like Receptors

Toll-like receptors (TLR) are essential to innate immunity for surveillance of pathogens. TLRs sense pathogen-associated molecular patterns (PAMPs) such as bacterial cell wall components and bacterial DNA and initiate the immune response. TLR2 and TLR4 have been shown to be especially important in the defense against *S. aureus*. TLR9 recognizes CpG motifs which are prevalent in bacterial DNA. In vitro, B cells from GPA patients stimulated by CpG and IL-2 produce higher levels of PR3-ANCA and MPO-ANCA (Hurtado et al. 2008). This was supported by an in vivo experiment in which mice were immunized with MPO and administered either a TLR2-ligand or TLR9-ligand. There was a clear enhancement of anti-MPO immunity and development of antibody-driven glomerular injury with either ligand (Summers et al. 2011). TLR2 is primarily involved in the promotion of the Th17 response, while TLR9 is important in the Th1 response and production of interferon-γ. In GPA, a shift toward a Th17 response has been

observed. These findings are supportive of a role of Toll-like receptors in the pathogenesis of GPA and suggest presence of a bacterial trigger. In a small series of 6 kidney biopsies from ANCA-positive GPA patients, there was evidence of increased TLR2, TLR4, and TLR9 compared to no expression of these molecules in disease controls (Batsford et al. 2011). Further studies of expression in TLRs in target tissues may provide further insight into pathogenesis.

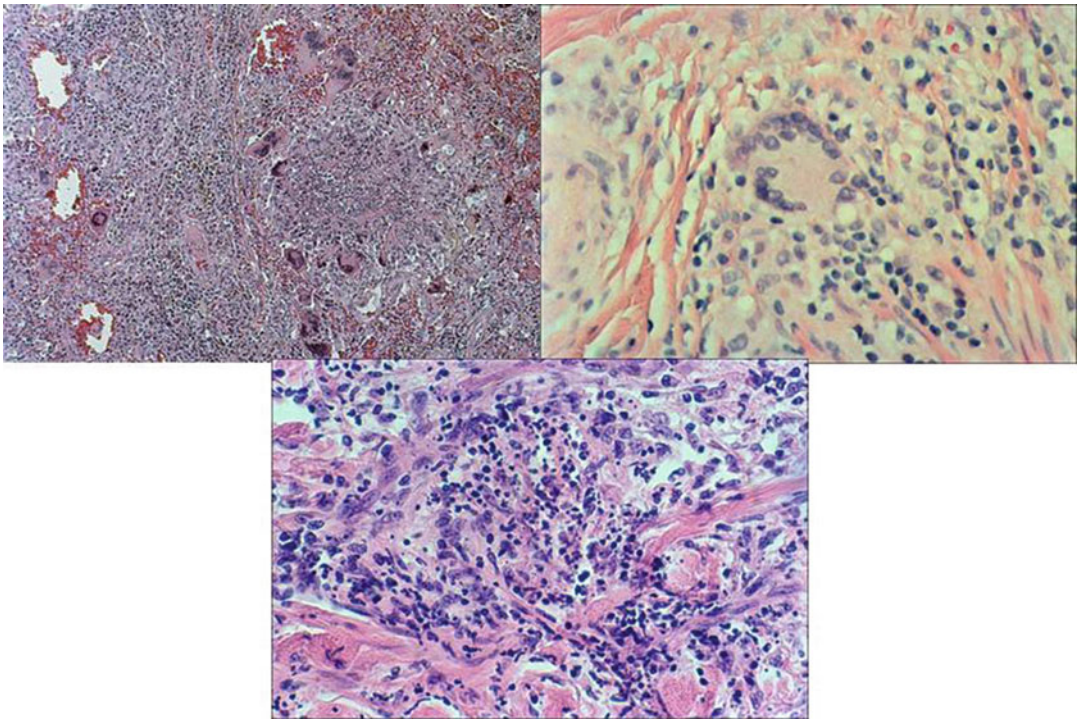
Granuloma Formation

GPA is much more commonly found to be associated with granuloma formation than other forms of small vessel vasculitis. Granulomas may be part of the inflammatory process in any tissue. The histological definition of a "granuloma" should not be used synonymously with a mass lesion or nodule and assumed to exclude the presence of vasculitis. In GPA, vasculitis may or may not be included in inflammatory lesions, whether or not granulomas are present. Granulomas are aggregates of mononuclear cells including activated macrophages, epithelioid cells (with or without giant cells) surrounded by T lymphocytes, B lymphocytes, and plasma cells. There may be an admixture of PMNs and eosinophils (Fig. 3). Thus, the cell content of a granuloma may be quite pleomorphic. It is the presence of activated macrophages and clustering of inflammatory cells that defines a granuloma. Granuloma formation is the body's response to poorly degraded intracellular pathogens (such as mycobacteria or fungi) or inert materials (silica or talc). Granuloma formation is common to sarcoidosis, Crohn's disease, Churg-Strauss syndrome, and Takayasu's arteritis. T lymphocytes, in response to antigen-presenting cells, are shifted toward a T-helper 1 (Th1) response. Th1 cells secrete high levels of IL-2, TNF-α, and interferon (IFN)-γ. This results in macrophage maturation and activation which can then form granulomas. Structurally, granulomas can appear similar to germinal centers seen in lymph nodes. This suggests that the granulomas may function similar to lymphoid organs in perpetuating the immune response. IL-12 is important in the differentiation of T cells into Th1 cells and is present in higher concentrations in patients with active and inactive GPA than controls. The presence of granulomas does not diminish a role for B cells and antibodies in GPA (Schilder 2010).

Clinical Features

Most patients (56–90 %) with GPA will develop otolaryngeal manifestations at some point during their disease (Wegener's Granulomatosis Etanercept Trial (WGET) Research Group 2005; Stone et al. 2010; Hoffman et al. 1992). The most common manifestations are inflammation of the sinuses and nasal mucosa. A history of recurrent sinus infections "refractory" to antibiotics is common. Visual exam of the nasal mucosa may reveal inflammation, ulcers, or nasal septal perforation. With chronic damage, the nasal mucosa may appear atrophic or "cobblestoning" may be present. Saddle nose deformities may appear with chronic damage of the nasal cartilaginous structures. Inflammation within the middle ear can be confused with infectious causes of otitis media. Hearing loss is most often conductive in nature; however, sensorineural deafness can also occur with cranial nerve VIII involvement. Eye manifestations may include conjunctivitis, episcleritis, retro-orbital mass, uveitis, scleritis, and retinal hemorrhages. Damage to the nasolacrimal ducts can lead to excessive tearing. Mouth sores are typically painful. Rarely, the gingiva can appear red, friable, and granular, giving the appearance of "strawberry gingiva." Subglottic stenosis may develop in approximately 10 % of patients (Wegener's Granulomatosis Etanercept Trial (WGET) Research Group 2005; Hoffman et al. 1992). Symptoms and signs of subglottic stenosis may include a history of difficulty with intubation, dyspnea in the absence of lung lesions, and stridor.

Pulmonary manifestations are present as an initial manifestation in up to half of patients; however, up to 85 % of patients eventually developing lung abnormalities. Pulmonary involvement may be asymptomatic or may



Vasculitis: Granulomatosis with Polyangiitis (Wegener's), Fig. 3 Lung biopsy from patient with GPA. (1) This lung biopsy contains areas of intense pleomorphic cellular inflammation and many giant cells. (2) Under higher power one can see an area of mostly mononuclear cell infiltrate with activated macrophages that have formed giant cells. Adjacent to these findings on the left side of the image is a necrotic vessel (vasculitis). (3) In another lung biopsy in GPA activated macrophages are part of a more pleomorphic population, including neutrophils, many of which have undergone lysis (leukocytoclasis)

present dramatically requiring ventilatory support. Frequent symptoms include cough, hemoptysis, and pleuritic chest pain. Diffuse alveolar hemorrhage requires aggressive treatment and if severe may lead to ventilator dependency. Endobronchial inflammation, stenosis, and ulcerations may occur as well. About one third of patients will have radiographs showing infiltrates or nodules without any specific symptoms. Therefore, it is important to screen patients with GPA for pulmonary disease.

Glomerulonephritis (GN) is a presenting manifestation in approximately 20 % of patients with GPA and will eventually affect 80 % of patients. The earliest sign of GN is asymptomatic microscopic hematuria and red blood cell casts in the urine. Kidney biopsies typically reveal necrotizing pauci-immune glomerulonephritis. Permanent kidney damage may develop rapidly. About 10 % of patients may develop end-stage renal disease (Hoffman et al. 1992; Villa-Forte et al. 2007). Patients receiving kidney transplantation have fewer relapses and a lower mortality compared to those who continue on dialysis. Graft survival in GPA is similar to that of other causes of end-stage kidney disease with 5-year graft survival reported as high as 93 % (Geetha et al. 2011). Microscopic hematuria and/or proteinuria may persist for years despite a sustained remission (Magrey et al. 2009; Geetha et al. 2012).

Skin manifestations will eventually develop in 46 % of patients with GPA and may include palpable purpura, ulcers, vesicles, papules, and subcutaneous nodules. Nervous system involvement may occur and most typically manifests as mononeuritis multiplex in about 15 % of patients. Central nervous system involvement is rare

(8 % of patients) and can include strokes, cranial nerve palsies, and diabetes insipidus. Pericarditis is the most frequent cardiac manifestation (about 6 % of patients). Rarely, the cardiac muscles or vessels can be involved (<2 %). Rare cases of mass lesions have been reported in the kidneys, adrenal glands, parotid glands, pulmonary artery, breast, urethra, cervix, vagina, and spleen (Hoffman et al. 1992; Carrington and Liebow 1966).

There is a high risk of venous thrombosis in GPA. The estimated incidence is around 7 per 100 person-years. This is similar to the incidence observed in those with a history of thrombosis in the general population. Systemic lupus erythematosus (SLE) is another autoimmune condition with a high risk of thrombosis largely because of the association with antiphospholipid antibody syndrome. GPA is estimated to have seven times the risk of thrombosis as in SLE. The vast majority (80 %) of thrombotic events occur during periods of active disease. The pathogenesis behind this risk is incompletely understood. Risk factors, such as the presence of anti-β 2 glycoprotein antibodies, anticardiolipin antibodies, factor V Leiden mutation, prothrombin gene mutation, and methylenetetrahydrofolate reductase mutations, have not been consistently found in association with GPA (Sebastian et al. 2007; Merkel et al. 2005).

Diagnosis

GPA most commonly affects the upper airways, lungs, and kidneys. It should be suspected in patients presenting with pulmonary-renal syndromes. However, diverse manifestations have been described, and any organ can be involved. Earlier literature has referred to a subclass of patients as "limited" GPA, meaning absence of renal involvement (Carrington and Liebow 1966). However, the term "limited" was later redefined as lacking severe critical organ disease or life-threatening manifestations (Wegener's Granulomatosis Etanercept Trial (WGET) Research Group 2005). These terms are only accurate at the point in time that they are applied, as disease features often change over time. Positive ANCA antibodies, particularly those directed against PR3, are supportive of the diagnosis. However, up to 20 % of patients with GPA are ANCA negative.

Treatment

Over the last 30 years, the treatment strategies for patients with GPA have changed dramatically. Goals of treatment include achieving a sustained remission, prevention of relapses, and avoiding drug toxicity. Most patients can achieve remission. Glucocorticoids (GCs) remain the cornerstone of treatment. However, GCs alone are frequently ineffective at maintaining improvement and cannot be used in high doses indefinitely. A second immunosuppressive should always be used to anchor remission and allow for gradual GC tapering. The choice of the second agent is largely based on the severity of the manifestations. Severe disease is generally defined as manifestations that threaten critical organs or is life-threatening (i.e., diffuse alveolar hemorrhage, rapidly progressive glomerulonephritis, nervous system involvement, or gastrointestinal ischemia, etc.). For severe manifestations, pulse-dose glucocorticoids should be considered. Both cyclophosphamide (CYC) and rituximab (RTX) have been shown to be equivalent for induction of remission. CYC can be used in either monthly intravenous (IV) pulse doses (e.g., 0.5–1 mg/m^2) or a daily oral dose (e.g., 2 mg/kg/day). Hemorrhagic cystitis is a potential side effect from CYC but is extremely rare when use is restricted to 3–4 months for remission induction. Mesna should be given with IV CYC, and aggressive oral hydration should be part of CYC use regardless of route of administration. Blood counts should be performed regularly to monitor for hemocytopenias while using CYC. Because long-term use of CYC over more than 1 year is associated with increased risk of malignancies, especially transitional cell carcinoma of the bladder, cumulative CYC exposure should be limited to 3–6 months (Falk and Hoffman 2007; Fauci et al. 1983). RTX is given as multiple IV infusions (1,000 mg IV for 2 doses 2 weeks apart or

375 mg/m^2 body surface area weekly for 4 doses). Potential side effects include infusion reactions, late-onset neutropenia, or progressive multifocal leukoencephalopathy (Stone et al. 2010). For non-severe manifestations of GPA, methotrexate (MTX) can be used as an induction agent ($\geq$15 mg/week) (De Groot et al. 2005). A randomized controlled trial regarding the role of plasmapheresis in the treatment of glomerulonephritis and diffuse alveolar hemorrhage is ongoing.

After patients have achieved remission maintenance, additional immunosuppressive agents may be required to avoid relapses. Both MTX and azathioprine (AZA) are equally effective at sustaining remission (Jayne et al. 2003; Pagnoux et al. 2008). There remains a high relapse rate even while on maintenance medications. However, the majority of these relapses occur as the maintenance agent is being tapered or stopped (Villa-Forte et al. 2007). MTX is contraindicated in those patients with a creatinine greater than 2.0 mg/dl. Mycophenolate mofetil has been shown to be less effective at maintenance of remission compared with AZA (Hiemstra et al. 2010); however, it remains an option in those with intolerance or contraindications to MTX and AZA. Studies are ongoing regarding maintenance therapy after induction of remission with RTX. Other medications used in small series of patients include leflunomide and IV immunoglobulins. In the Wegener's Granulomatosis Etanercept (WGET) Trial, etanercept did not show efficacy when added to traditional treatment (CYC or MTX) (Wegener's Granulomatosis Etanercept Trial (WGET) Research Group 2005).

Local Therapy

Certain manifestations of GPA may require local therapy. Damage may occur to the nasal mucosa and paranasal sinus structures and include symptoms such as mucopurulent rhinorrhea, nasal crusting, epistaxis, facial pain, and anosmia. Daily nasal irrigation regimens including a lubricant or emollient in addition to a daily saline rinse can help reduce dryness and help remove crusts. Saddle nose deformities can occur in up to 24 % of patients. Surgical correction should be delayed until the patient has been in remission for a lengthy period of time. Prosthetic material is generally not recommended as it can increase the risk of infection, and active disease can threaten the vitality of the prosthesis.

The nasolacrimal duct can also be damaged and lead to epiphora or dacryocystitis. Surgical dacryocystorhinostomy during a period of remission can improve these symptoms.

Retro-orbital masses can lead to diplopia, proptosis, ocular movement limitations, and vision loss. Systemic therapy with glucocorticoids and CYC is often of modest (or no) benefit for orbital mass lesions; however, there are reports of improvement with RTX. The orbital mass often evolves into a pannus of fibrous tissue that can lead to an orbital contracture. This can produce enophthalmos, ocular movement limitations, and vision loss. Refractory disease may require surgical decompression.

Subglottic stenosis requires laryngoscopic and/or bronchoscopic evaluation to determine if the lesion is actively inflamed or has become a rind of scar tissue. Active subglottic inflammation can be resistant to systemic immunosuppressive agents in up to 80 % of patients. Local therapy includes intralesional glucocorticoids in combination with use of graduated dilators. Multiple topical applications of mitomycin C may be used to diminish fibrosis and restenosis. However, this intervention has not been studied in a randomized controlled fashion. Local laser therapy should be avoided as it can add to enhancement of scar tissue and high rates of restenosis. Refractory tracheobronchial disease may require repeated local dilatation, with intralesional depo-glucocorticoid injections (Hernandez-Rodriguez et al. 2010).

Prophylaxis

Prophylactic measures should be used to avoid treatment-related adverse events. *Pneumocystis jirovecii* pneumonia (PJP) has been reported to occur in 6 % of GPA patients who do not receive chemoprophylaxis. Trimethoprim-sulfamethoxazole (T/S) (160/800 mg given three times weekly or 80/400 mg given daily) will prevent most cases of PJP. Low-dose T/S

prophylaxis can safely be given to patients on MTX; however, full doses of these two folate antagonists should be avoided, as they can lead to bone marrow failure. Patients with contraindications or allergies to sulfa drugs can be given dapsone 100 mg daily or atovaquone 1,500 mg daily. Monthly inhaled pentamidine 300 mg can also be used but may not offer the same degree of protection. High-dose glucocorticoids can predispose patients to osteoporosis. Daily calcium and vitamin D supplements should be considered on all patients on glucocorticoids. In addition, bisphosphonates should be considered for osteoporosis prevention. Appropriate vaccinations should be considered in GPA patients and family members including the yearly influenza vaccine and the pneumococcal vaccine. Care should be taken to avoid live vaccines in patients using immunosuppressive medications. Daily folic acid or weekly folinic acid replacement can help prevent mucosal and hepatic side effects from MTX. Contraception should be emphasized in both male and female patients while being treated with potentially teratogenic medications. Depot leuprolide can reduce the rate of premature ovarian failure in female patients treated with CYC.

Conclusion

The pathogenesis of GPA is incompletely understood. It is categorized as an ANCA-associated vasculitis, emphasizing the importance of B cells and antibodies. While ANCA has a prominent role in GPA, it should be recognized that up to 20 % of patients with a convincing clinical phenotype for GPA are ANCA negative. Furthermore, ANCA levels correlate poorly with relapse and disease activity in large trials. Mononuclear cells that are of both Th1 and Th2 lineage are involved in a complex cross talk with B cells and neutrophils. The key to understanding the pathogenesis of GPA lies in discovering its etiology. That almost all GPA patients have evidence of airway disease with or without systemic symptoms at onset is suggestive of an infectious, airborne trigger. Increasing circumstantial evidence points to an infectious trigger in GPA.

Cross-References

- ▶ CTLA4-Ig
- ▶ Eosinophilic Granulomatosis with Polyangiitis (Churg-Strauss Syndrome)
- ▶ Immune System and Kidney
- ▶ Indications for Biopsy in Autoimmune GN
- ▶ Neutrophils in Endothelial Damage
- ▶ PTPN22
- ▶ Vasculitis and the Kidney
- ▶ Vasculitis: Granulomatosis with Polyangiitis (Wegener's)

References

Batsford S, Duermueller U, Seemayer C, Mueller C, Hopfer H, Mihatsch M. Protein level expression of Toll-like receptors 2, 4 and 9 in renal disease. Nephrol Dial Transplant. 2011;26(4):1413–6.

Bonaci-Nikolic B, Andrejevic S, Pavlovic M, Dimcic Z, Ivanovic B, Nikolic M. Prolonged infections associated with antineutrophil cytoplasmic antibodies specific to proteinase 3 and myeloperoxidase: diagnostic and therapeutic challenge. Clin Rheumatol. 2010;29(8):893–904.

Carrington CB, Liebow A. Limited forms of angiitis and granulomatosis of Wegener's type. Am J Med. 1966;41(4):497–527.

Cotch MF, Hoffman GS, Yerg DE, Kaufman GI, Targonski P, Kaslow RA. The epidemiology of Wegener's granulomatosis. Estimates of the five-year period prevalence, annual mortality, and geographic disease distribution from population-based data sources. Arthritis Rheum. 1996;39(1):87–92.

De Groot K, Rasmussen N, Bacon PA, Tervaert JW, Feighery C, Gregorini G, et al. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2005;52(8):2461–9.

Falk RJ, Hoffman GS. Controversies in small vessel vasculitis–comparing the rheumatology and nephrology views. Curr Opin Rheumatol. 2007;19(1):1–9.

Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. Ann Intern Med. 1983;98(1):76–85.

Geetha D, Eirin A, True K, Valentina Irazabal M, Specks U, Seo P, et al. Renal transplantation in antineutrophil cytoplasmic antibody-associated vasculitis: a multicenter experience. Transplantation. 2011;91(12):1370–5.

Geetha D, Seo P, Ellis C, Kuperman M, Levine SM. Persistent or new onset microscopic hematuria in

patients with small vessel vasculitis in remission: findings on renal biopsy. J Rheumatol. 2012;39(7):1413–7.

Gencik M, Meller S, Borgmann S, Fricke H. Proteinase 3 gene polymorphisms and Wegener's granulomatosis. Kidney Int. 2000;58(6):2473–7.

Giscombe R, Wang X, Huang D, Lefvert AK. Coding sequence 1 and promoter single nucleotide polymorphisms in the CTLA-4 gene in Wegener's granulomatosis. J Rheumatol. 2002;29(5):950–3.

Gupta AK, Joshi MB, Philippova M, Erne P, Hasler P, Hahn S, Resink TJ. Activated endothelial cells induce neutrophil extracellular traps and are susceptible to NETosis-mediated cell death. FEBS Lett. 2010;584(14):3193–7.

Hernandez-Rodriguez J, Hoffman GS, Koening CL. Surgical interventions and local therapy for Wegener's granulomatosis. Curr Opin Rheumatol. 2010;22(1):29–36.

Hewins P, Belmonte F, Charles Jennette J, Falk RJ, Preston GA. Longitudinal studies of patients with ANCA vasculitis demonstrate concurrent reactivity to complementary PR3 protein segments cPR3m and cPR3C and with no reactivity to cPR3N. Autoimmunity. 2011;44(2):98–106.

Hiemstra TF, Walsh M, Mahr A, Savage CO, de Groot K, Harper L, et al. Mycophenolate mofetil vs azathioprine for remission maintenance in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled trial. JAMA. 2010;304(21):2381–8.

Hoffman GS, Kerr GS, Leavitt RY, Hallahan CW, Lebovics RS, Travis WD, et al. Wegener granulomatosis: an analysis of 158 patients. Ann Intern Med. 1992;116(6):488–98.

Hurtado PR, Jeffs L, Nitschke J, Patel M, Sarvestani G, Cassidy J, et al. CpG oligodeoxynucleotide stimulates production of anti-neutrophil cytoplasmic antibodies in ANCA associated vasculitis. BMC Immunol. 2008;9:34.

Jagiello P, Gencik M, Arning L, Wieczorek S, Kunstmann E, Csernok E, et al. New genomic region for Wegener's granulomatosis as revealed by an extended association screen with 202 apoptosis-related genes. Hum Genet. 2004;114(5):468–77.

Jagiello P, Aries P, Arning L, Wagenleiter SE, Csernok E, Hellmich B, et al. The PTPN22 620W allele is a risk factor for Wegener's granulomatosis. Arthritis Rheum. 2005;52(12):4039–43.

Jayne D, Rasmussen N, Andrassy K, Bacon P, Tervaert JW, Dadoniene J, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med. 2003;349(1):36–44.

Kain R, Exner M, Brandes R, Ziebermayr R, Cunningham D, Alderson CA, et al. Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. Nat Med. 2008;14(10):1088–96.

Kain R, Tadema H, McKinney EF, Benharkou A, Brandes R, Peschel A, et al. High prevalence of autoantibodies to hLAMP-2 in anti-neutrophil cytoplasmic antibody-associated vasculitis. J Am Soc Nephrol. 2012;23(3):556–66.

Kallenberg CG. Pathogenesis of PR3-ANCA associated vasculitis. J Autoimmun. 2008;30(1–2):29–36.

Kessenbrock K, Krumbholz M, Schonermarck U, Back W, Gross WL, Werb Z, et al. Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med. 2009;15(6):623–5.

Knight A, Sandin S, Askling J. Risks and relative risks of Wegener's granulomatosis among close relatives of patients with the disease. Arthritis Rheum. 2008;58(1):302–7.

Knight A, Sandin S, Askling J. Occupational risk factors for Wegener's granulomatosis: a case-control study. Ann Rheum Dis. 2010;69(4):737–40.

Lane SE, Watts RA, Bentham G, Innes NJ, Scott DG. Are environmental factors important in primary systemic vasculitis? A case-control study. Arthritis Rheum. 2003;48(3):814–23.

Magrey MN, Villa-Forte A, Koening CL, Myles JL, Hoffman GS. Persistent hematuria after induction of remission in Wegener granulomatosis: a therapeutic dilemma. Medicine (Baltimore). 2009;88(6):315–21.

Merkel PA, Lo GH, Holbrook JT, Tibbs AK, Allen NB, Davis Jr JC, et al. Brief communication: high incidence of venous thrombotic events among patients with Wegener granulomatosis: the Wegener's clinical occurrence of thrombosis (WeCLOT) study. Ann Intern Med. 2005;142(8):620–6.

Morris H, Morgan MD, Wood AM, Smith SW, Ekeowa UI, Herrmann K, et al. ANCA-associated vasculitis is linked to carriage of the Z allele of alpha(1) antitrypsin and its polymers. Ann Rheum Dis. 2011;70(10):1851–6.

Pagnoux C, Mahr A, Hamidou MA, Boffa JJ, Ruivard M, Ducroix JP, et al. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. N Engl J Med. 2008;359(26):2790–803.

Popa ER, Stegeman CA, Abdulahad WH, van der Meer B, Arends J, Manson WM, et al. Staphylococcal toxic-shock-syndrome-toxin-1 as a risk factor for disease relapse in Wegener's granulomatosis. Rheumatology (Oxford). 2007;46(6):1029–33.

Pudifin DJ, Duursma J, Gathiram V, Jackson TF. Invasive amoebiasis is associated with the development of anti-neutrophil cytoplasmic antibody. Clin Exp Immunol. 1994;97(1):48–51.

Raynauld JP, Bloch DA, Fries JF. Seasonal variation in the onset of Wegener's granulomatosis, polyarteritis nodosa and giant cell arteritis. J Rheumatol. 1993;20(9):1524–6.

Schilder AM. Wegener's Granulomatosis vasculitis and granuloma. Autoimmun Rev. 2010;9(7):483–7.

Sebastian JK, Voetsch B, Stone JH, Romay-Penabad Z, Lo GH, Allen NB, et al. The frequency of anticardiolipin antibodies and genetic mutations associated with hypercoagulability among patients with

Wegener's granulomatosis with and without history of a thrombotic event. J Rheumatol. 2007;34(12):2446–50.
Stegeman CA, Tervaert JW, de Jong PE, Kallenberg CG. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. N Engl J Med. 1996;335(1):16–20.
Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med. 2010;363(3):221–32.
Summers SA, Steinmetz OM, Gan PY, Ooi JD, Odobasic D, Kitching AR, et al. Toll-like receptor 2 induces Th17 myeloperoxidase autoimmunity while Toll-like receptor 9 drives Th1 autoimmunity in murine vasculitis. Arthritis Rheum. 2011;63(4):1124–35.
van der Geld YM, Stegeman CA, Kallenberg CG. B cell epitope specificity in ANCA-associated vasculitis: does it matter? Clin Exp Immunol. 2004;137(3):451–9.
Villa-Forte A, Clark TM, Gomes M, Carey J, Mascha E, Karafa MT, et al. Substitution of methotrexate for cyclophosphamide in Wegener granulomatosis: a 12-year single-practice experience. Medicine (Baltimore). 2007;86(5):269–77.
Wegener F. Thoughts and observations of a pathologist. Eur Arch Otorhinolaryngol. 1990;247:133–42.
Wegener's Granulomatosis Etanercept Trial (WGET) Research Group. Etanercept plus standard therapy for Wegener's granulomatosis. N Engl J Med. 2005;352(4):351–61.
Willcocks LC, Lyons PA, Rees AJ, Smith KG. The contribution of genetic variation and infection to the pathogenesis of ANCA-associated systemic vasculitis. Arthritis Res Ther. 2010;12(1):202.
Zhou Y, Huang D, Paris PL, Sauter CS, Prock KA, Hoffman GS. An analysis of CTLA-4 and proinflammatory cytokine genes in Wegener's granulomatosis. Arthritis Rheum. 2004;50(8):2645–50.

Vasculitis: Henoch-Schönlein Purpura

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Synonyms

Anaphylactoid purpura; Purpura rheumatica

Definition

Henoch-Schönlein Purpura (HSP) belongs to the group of diseases known as systemic or leukocytoclastic vasculitides. HSP is a multisystem disorder involving the skin, joints, gastrointestinal tract, and kidneys which mainly affects children. Its course is typically benign and self-limited though significant long-term complications may arise in a small percentage of patients.

The classic triad of symptoms in HSP includes non-thrombocytopenic palpable purpura, arthritis, and colicky abdominal pain. Renal involvement, if present, is often mild but rarely may progress to chronic renal failure. Involvement of other organ systems occurs less commonly.

Pathogenesis

HSP is an immunoglobulin A (IgA)-mediated immune vasculitis but the exact etiology remains unknown. As with most immune-mediated diseases, a combination of genetic, immunologic, and environmental factors is implicated (Saulsbury 1999). HSP is most often triggered by exposure to an exogenous antigen, typically an upper respiratory pathogen, immunization, or medication.

IgA plays a central role in the development of HSP. Normally, IgA is found in mucosal fluids and serum and exists at 2 subtypes: IgA1 and IgA2. Mucosal IgA is 60 % IgA2 and mainly polymeric, whereas serum IgA is mainly IgA1 and monomeric. In HSP, immune complexes contain polymeric IgA1 which is capable of activating the alternate complement pathway. During the course of the illness, total serum hemolytic complement (CH_{50}) and properdin levels may be depressed, while levels of C3d, a C3 breakdown product, may be elevated. Immune complex deposition incites release of inflammatory mediators, with infiltration of polymorphonuclear leukocytes and monocytes. The end result is tissue injury mediated by alternative complement pathway activation and membrane attack complex formation. Some authors have proposed

that abnormal glycosylation of the IgA leads to deposition in the renal mesangium and subsequent development of HSP. An abnormal form of the IgA1 known as Gal-d IgA1 has been identified and found to be higher in HSP nephritis compared with HSP without nephritis and normal controls (Lau et al. 2007). IgA autoantibodies, including rheumatoid factor and antineutrophil cytoplasmic antibodies (ANCA), also may be found in serum of patients with HSP, though their role in pathogenesis of disease is unclear (Boulis et al. 2010).

Histology is consistent with an immune complex-mediated leukocytoclastic vasculitis. Small blood vessels demonstrate necrosis associated with a perivascular infiltrate composed of neutrophils and mononuclear cells. Immunofluorescence and electron microscopy of fresh lesions may detect deposits of IgA, C3, and fibrin (Davin 2011). Renal biopsies typically show an endocapillary proliferative glomerulonephritis involving endothelial and mesangial cells. Proliferation of extracapillary cells and fibrin deposition in Bowman's capsule may also occur, resulting in variable degrees of crescent formation.

Epidemiology

HSP is the most common form of childhood vasculitis, with an incidence of 10 to 20 per 100,000 children less than age 17 years and a peak age of onset between 4 and 6 years (Gardner-Medwin et al. 2002). Most patients are between the ages of 3 and 10 years, boys are affected 1.5–2 times as frequently as girls, and HSP is most prevalent during the fall, winter, and spring, periods of time coinciding with higher rates of infection. These associations provide support for an infectious trigger. Indeed, about 50 % of patients with HSP have a preceding upper respiratory tract infection, most commonly with Streptococcus. Other reported triggers for HSP include Staphylococcus aureus, Haemophilus influenzae, influenza, parainfluenza, Epstein-Barr virus, adenovirus, parvovirus, mycoplasma, vaccinations, drugs, and insect bites (Levy et al. 1976). Interestingly, in adults, HSP seems to be more common in the summer, which suggests a different etiopathogenesis.

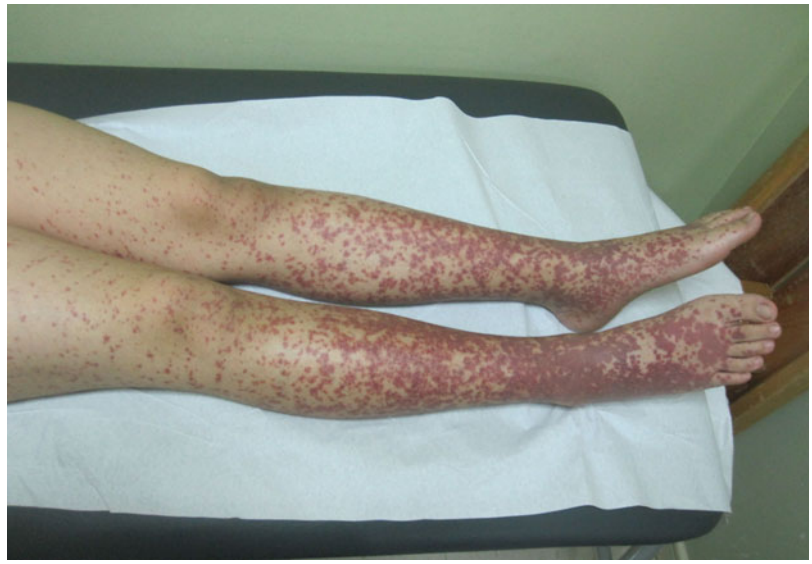
The incidence of HSP is also affected by a patient's background genetic makeup. For example, the rate of HSP is as high as 5 % in patients with a mutation of the gene associated with familial Mediterranean fever (known as the "FEMV" gene) (Bayram et al. 2011) and in those with certain complement deficiencies.

Clinical Manifestations

The classic presentation is that of a previously well child developing palpable purpura, arthritis or arthralgia, and colicky abdominal pain. Occasionally, these may be preceded by malaise or a low-grade fever. These symptoms may develop in rapid succession or sequentially over a period of days to weeks. **Rash** is the initial feature in about 75 % of affected children. It is present in almost all children with the disease, though this is at least partially due to the fact that the diagnosis is exceedingly difficult without evidence of palpable purpura.

The typical rash is a deep purple or dark red, non-blanching papular exanthem that is painless and non-pruritic (Fig. 1). It often begins as urticarial wheals or erythematous papules that eventually evolve to form the characteristic purpura or ecchymoses. Patients also may develop petechiae. Occasionally, areas of skin may demonstrate bullous or hemorrhagic lesions, or areas that look like deep bruises. The purpura generally resolves over the course of a week, fading to yellow or purple brown as the heme pigment is phagocytosed and enzymatically converted to bilirubin and then hemosiderin. Successive crops of rash may appear over time, giving the rash a polymorphous appearance. Episodes of rash recur in approximately one third of patients, usually within weeks, but at times up to a year after disease onset. Successive recurrences tend to become progressively milder, and the rash does not scar.

The rash of HSP is generally distributed symmetrically. In younger, non-ambulatory children, gravity-dependent areas such as the



Vasculitis: Henoch-Schönlein Purpura, Fig. 1 Typical dependent purpuric rash of Henoch-Schönlein purpura (Courtesy of Arturo Borzutzky, M.D.)

face, trunk, and upper extremities predominate, while pressure-dependent areas such as extensor surfaces of the legs and buttocks are more commonly affected in older patients. This pattern may reflect deposition of large IgA immune complexes which then activate the alternate complement pathway, disrupting vascular integrity and leading to the rash. Other signs of dermatologic involvement include localized subcutaneous edema which may be slightly tender. This typically affects the scalp, forehead, periorbital areas, or the dorsal aspects of the hands and feet.

Joint involvement is the second most common manifestation of HSP. **Arthralgias** or **arthritis** occurs in 50–90 % of all affected children and is the presenting symptom in about one in six patients (Trapani et al. 2005). Arthritis is nondestructive with no permanent sequelae. There is often prominent periarticular swelling and tenderness, but usually without joint effusion, erythema, or warmth. Typically, one to three joints are affected, the knees and ankles being the most common. Elbows, wrists, and hands may be affected as well. Small joint involvement is rare, though edema of the hands and feet may be confused with interphalangeal joint synovitis. Regardless of the number of joints involved, they tend to be very uncomfortable, and patients may have considerable pain and limitation of motion as a result.

The third cardinal manifestation of HSP is gastrointestinal involvement, most often **colicky abdominal pain** (50–75 % of patients). Abdominal symptoms usually occur within a week of the rash but may present up to 4 weeks later. When abdominal pain precedes the rash, it may be difficult to distinguish from appendicitis.

Abdominal pain is caused by submucosal hemorrhage and edema of the gastrointestinal (GI) tract, analogous to superficial manifestations of HSP visible in the integument. It is often self-limited but may be severe enough to prevent adequate oral intake. The pain typically waxes and wanes, with exacerbations causing affected children to curl up or cry, followed by a pain-free interval minutes later. Any acute change in the nature or intensity of the pain should prompt urgent evaluation for complications such as intussusception, bowel infarction, or perforation.

Intussusception is the most common gastrointestinal complication of HSP, occurring in 1–5 % of patients. The lead point of the intussusception is hemorrhage and edema in a portion of the gut. Such lesions are ileoileal in up to 60 % of cases, in contrast to the typical ileocolic intussusception accompanying mesenteric adenitis or nonspecific viral infections in young children (Choong and Beasley 1998). When intussusception is suspected, ultrasonography should be the

initial screening test. Contrast enemas, on the other hand, are unable to detect the ileoileal intussusception seen in HSP because the contrast does not reach the ileum.

Gastrointestinal bleeding may manifest as melena, heme-positive stools, or hematemesis. Massive gastrointestinal hemorrhage is rare. Vomiting and paralytic ileus may be seen. Rarer gastrointestinal manifestations include acute pancreatitis, cholecystitis, hydrops of the gall bladder, or protein-losing enteropathy (Feldt and Stickler 1962).

Renal involvement represents the fourth classical feature of HSP, seen in 20–54 % of children (Brogan and Bagga 2011). Renal involvement is often asymptomatic – detected only on urinalysis – and usually occurs within the first 6 weeks of disease (Stewart et al. 1988). The overwhelming majority of patients develop some evidence of nephritis during the first 6 months of the illness, with children most commonly demonstrating microscopic hematuria with or without mild proteinuria. The risk of progression to chronic renal failure in such cases is less than 5 %. The risk rises to 19 % if the child has both nephritic and nephrotic features (Narchi 2005). While older patients have approximately the same overall incidence of renal involvement, the likelihood of progression to renal failure is significantly higher – up to 30 % in adults. Renal biopsies show histological features similar to IgA nephropathy, including crescent formation in the most severe cases.

Although the skin, joints, GI tract, and kidneys are most commonly involved in HSP, as a systemic vasculitis it may affect virtually any organ system. In one study, pulmonary involvement was detected by measurement of pulmonary diffusion capacity in 97 % of children with HSP (Chaussain et al. 1992), though subsequent attempts to confirm these findings were unsuccessful. Interstitial pneumonia and diffuse alveolar hemorrhage have been described, mostly in adults and adolescents, but for the most part pulmonary involvement is asymptomatic.

Clinically relevant neurologic involvement is rare and mostly affects patients with significant renal disease. Seizures, focal neurologic deficits, ataxia, mental status changes, headaches, and peripheral neuropathies have all been described (Garzoni et al. 2009). Such central nervous system dysfunction may results from a variety of causes, including severe hypertension, electrolyte aberrations due to GI and renal involvement, or stroke and intracranial hemorrhage. Only in the latter cases are symptoms persistent.

Other rarer findings in HSP may include scrotal swelling and tenderness, usually from epididymo-orchitis in 5 % (Brogan and Bagga 2011) and hypocomplementemia from vascular deposition of activated immune complexes.

Diagnosis and Evaluation

The diagnosis of HSP is a clinical one, often made after the appearance of the typical rash, with associated articular or gastrointestinal involvement in a child. There is no specific laboratory test for HSP. Rather, investigations serve the purpose of assessing the extent of organ involvement or excluding alternative diagnoses. Routine blood tests typically yield nonspecific abnormalities. A complete blood count may reveal a raised white blood cell and platelet count. A mild anemia may reflect occult gastrointestinal hemorrhage or anemia associated with inflammation. Acute phase reactants may be somewhat increased, with the degree of elevation often related to the triggering antigen. Thus, HSP related to a streptococcal infection is typically associated with higher inflammatory markers than one precipitated by a viral illness. Coagulation studies to exclude bleeding diatheses are not strictly necessary if the clinical picture is typical, though they may be part of the process of excluding sepsis. In uncomplicated HSP, the prothrombin and partial thromboplastin times should be normal. Serum IgA is elevated in 50–70 % of patients with HSP (Trygstad and Stiehm 1971). Such a finding is not typically seen in other causes of purpura, abdominal pain, hematuria, or arthritis, so an elevated IgA level may support the diagnosis of HSP. Serum complement levels are normal in about 90 % of patients, while autoantibodies

are usually absent and should not be routinely interrogated unless the diagnosis of HSP is in question.

Urinalysis for cells, casts, and proteinuria should be carried out at diagnosis in all patients. All adults should have a serum creatinine measured at presentation as they are at increased risk for significant renal disease, whereas in children, this is not necessary if the child is normotensive and urinalysis is bland. Evidence of renal involvement usually follows other manifestations of HSP, so testing should be continued for at least 6 months after the acute presentation.

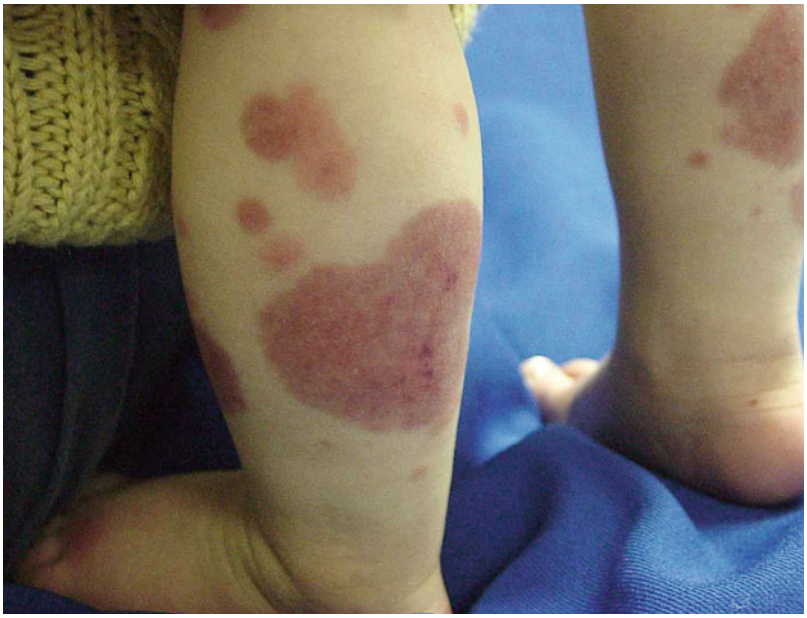
In ambiguous cases in which the presentation is atypical or incomplete, biopsy of an affected organ (preferably a skin lesion that is less than 24 h old) may be helpful, demonstrating a leukocytoclastic vasculitis with IgA deposits in the postcapillary venules. Similar findings are found in mesangial vessels if the kidney is biopsied. As HSP is significantly less prevalent in adults, biopsies may be more crucial for confirmation of the diagnosis in older patients, particularly to distinguish the condition from other vasculitides. A renal biopsy is indicated in the presence of significant proteinuria, renal impairment, or persistent evidence of glomerulonephritis (hematuria, especially with dysmorphic red blood cells or red blood cell casts, and/or hypertension). Histology is useful for delineating extent and severity of nephritis and hence determining treatment. The risk of developing end stage renal disease increases with the percentage of glomeruli with crescents.

When abdominal symptoms are present and persistent, imaging should be carried out to look for complications such as intussusception, ileus, ischemia, or bowel perforation. Plain radiographs may show dilated loops of bowel from obstruction or ileus. Abdominal ultrasonography is the imaging of choice for suspected intussusception. The classic finding of a "target/bull's eye/doughnut sign" on transverse section (appearance of alternating layers of hyper- and hypoechoic lesions) results from one portion of bowel invaginating into another. Ultrasound may also reveal bowel wall thickening, abnormal peristalsis, or hematomas. Imaging is indicated as well in boys with HSP who develop scrotal pain. Doppler flow studies will help distinguish testicular torsion, in which blood flow is decreased or absent, from the epididymo-orchitis of HSP, characterized by perfusion that is normal or increased as a result of inflammation.

Differential Diagnosis

The diagnosis of HSP is usually straightforward, particularly in children, unless the presentation is incomplete or atypical, or dermatologic features are initially absent. Nonetheless, other causes of palpable purpura must be excluded before a patient with presumed HSP can be confirmed to have a benign condition. The differential diagnosis of cutaneous manifestations of HSP, particularly petechiae and purpura, includes septicemia, idiopathic thrombocytopenic purpura, hemolytic-uremic syndrome, leukemia, and coagulopathies (e.g., hemophilia). They are not always clinically distinguishable from HSP but may be suggested by the presence of abnormal platelet counts and coagulation studies.

When purpura is accompanied by normal platelet counts and coagulation studies, considerations mainly include other vasculitides, including those associated with connective tissue disorders such as systemic lupus erythematosus (SLE). Most common of the primary vasculitides is hypersensitivity (or allergic) vasculitis, an inflammatory condition of small vessels primarily seen in adults following drug exposure or infection. The patient typically presents with fever, urticaria, lymphadenopathy, arthralgias, elevated ESR, and depressed serum complement. Histology shows a leukocytoclastic vasculitis without IgA deposition in vessel walls. Vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA), such as Churg-Strauss syndrome (CSS) and granulomatosis with polyangiitis (Wegener's) (GPA), is quite rare but delays in diagnosis may lead to development of irreversible renal or pulmonary failure. Children younger than 2 years of age may develop an immune complex-mediated vasculitis known as acute hemorrhagic edema of infancy (AHEI).



Vasculitis: Henoch-Schönlein Purpura, Fig. 2 Large non-blanching macules typical of acute hemorrhagic edema of infancy (AHEI)

This is likely a forme fruste of HSP that develops in younger patients unable to mount an IgA response to foreign antigens (Saraclar et al. 1990). The rash of AHEI resembles that of HSP though the purpuric lesions are often larger than the typical rash of HSP (Fig. 2). It is usually associated with fever and generalized edema, but renal involvement is typically absent.

Etiologic considerations in patients presenting with renal disease are quite broad and beyond the scope of this entry. Histopathologically, the glomerulonephritis of HSP is most similar to, and in fact may be indistinguishable from, that seen in patients with IgA nephropathy or Berger's disease.

Management

HSP is for the most part benign and self-limited, with therapy focused on providing symptomatic relief and supportive care. Hospitalization may be necessary for patients who are unable to maintain adequate oral hydration and have severe abdominal or joint pain, significant gastrointestinal bleeding, altered mental status, renal insufficiency, hypertension, or significant nephritis or nephrotic syndrome.

Joint pain tends to respond to acetaminophen, with severe symptoms improving after nonsteroidal anti-inflammatory drugs (NSAIDs), e.g., naproxen 10–20 mg/kg/day (maximum 1,500 mg, 1,000 mg if used beyond a week) in two divided doses. There is no contraindication to this in the setting of normal renal function and/or urinalysis and in particular no evidence that NSAIDs increase the risk of gastrointestinal hemorrhage in HSP. Nevertheless, they should be used with caution, while maintaining adequate hydration, to minimize potential adverse effects. Abdominal pain also may be ameliorated by acetaminophen and/or NSAIDs. Patients with physical findings suggestive of peritonitis or obstruction should undergo urgent evaluation, often by a surgeon for concerns of bowel infarction, perforation, or intussusception. Physical examination alone is often inadequate to diagnose intestinal vasculitis.

Corticosteroids may be helpful in cases characterized by more significant discomfort. These medications have not been shown to shorten the duration of abdominal involvement or reduce the risk of intussusception (Weiss et al. 2007). However, they may be indicated in patients who have not responded to NSAIDs and whose abdominal pain is severe enough to

prevent adequate oral intake or whose joint involvement interferes with ambulation and activities of daily living. Steroids should not be given until abdominal complications such as intussusception or perforation are definitively excluded, as they may mask signs of peritonitis. It is also important to stress the fact that steroids do not alter the pathophysiology of the disease, but only mitigate associated inflammation. Thus, after starting prednisone or methylprednisolone at a dose of 1–2 mg/kg/day, in two divided doses, steroids should be tapered gradually, by no more than 25 % per week. Tapering more rapidly than the speed with which pathologic immune complexes are cleared from the circulation may increase the risk of symptom recurrence. Additionally, bowel edema may compromise absorption of medications, including steroids, and so parenteral administration may be required until acute intestinal involvement improves. Preemptive use of steroids has not been consistently shown to prevent development of long-term sequelae or alter the prognosis of HSP, so such use of steroids is generally not recommended.

More potent immunosuppression with agents such as cyclophosphamide, azathioprine, or mycophenolate mofetil may be necessary in children with biopsy-proven crescenteric glomerulonephritis or organ- and/or life-threatening complications such as pulmonary hemorrhage or central nervous system vasculitis. Reports of success with these medications are limited to case series. Guidelines for their use are not available and treatment courses are typically complex and prolonged. Thus, involvement of specialists experienced in the use of potent immunosuppressive medications for the treatment of severe inflammatory diseases is essential in such cases.

Prognosis

The prognosis of HSP in children is generally excellent. Apart from renal disease, the mean duration of symptoms is 3–4 weeks. Approximately one third of children have at least one recurrence (Saulsbury 2002), usually within 4 months of the initial episode, although recurrences may occur up to 2 years later. Recurrences are more likely in those who have a more severe course of HSP, though subsequent episodes tend to be successively milder and of shorter duration.

Renal involvement determines the long-term prognosis of HSP and may cause late morbidity and mortality. The risk of progressive renal disease is highest in adults, with severity of renal involvement highest in patients with nephritic range proteinuria, elevated serum creatinine, hypertension, and severe histologic findings. Generally, chronic kidney disease is reported in 1–5 % of children with HSP, with the higher estimates likely affected by ascertainment bias in case series from tertiary referral centers.

Monitoring

Generally, patients with HSP should have urinalysis and blood pressure monitoring weekly to biweekly for the first 2 months after presentation. Somewhat less frequent follow-up should continue for up to a year thereafter. If blood pressure and urine sediment remain normal, ongoing monitoring may be limited to measurement of blood pressure and urinalysis during annual physical examinations. Serum creatinine should be followed more closely in any patient with persistent or significant urinary abnormalities or hypertension, often in conjunction with a nephrologist. Pregnant women with a history of HSP should be monitored closely, as they have a higher risk of hypertension and proteinuria (Ronkainen et al. 2002). Neither a strong family history of HSP nor a previous episode of HSP seems to confer an increased risk of developing HSP. Similarly, repeated development of apparently unrelated episodes of HSP is rare; such patients may recover following removal of indwelling hardware or other antigenic triggers. If no trigger can be identified, colchicine or intravenous immunoglobulin may ameliorate exacerbations.

Cross-References

- ▶ IgA Nephropathy
- ▶ Vasculitis and the Kidney

References

Bayram C, et al. Prevalence of MEFV gene mutations and their clinical correlations in Turkish children with Henoch-Schönlein purpura. Acta Paediatr. 2011;100:745.

Boulis E et al. Adult-onset Henoch-Schonlein purpura with positive c-ANCA (anti-proteinase 3): case report and review of literature. Rheumatol Int. 2013;33(2):493–6. doi:10.1007/s00296-010-1608-x. Epub 2010 Oct 21.

Brogan P, Bagga A. Leukocytoclastic vasculitis. In: Cassidy JT, Petty RE, Laxer RM, et al., editors. Textbook of pediatric rheumatology. Philadelphia: Saunders Elsevier; 2011. p. 482–97.

Chaussain M, et al. Impairment of lung diffusion capacity in Schönlein-Henoch purpura. J Pediatr. 1992;121(1):12.

Choong CK, Beasley SW. Intra-abdominal manifestations of Henoch-Schönlein purpura. J Paediatr Child Health. 1998;34:405.

Davin J-C. Henoch-Schonlein purpura nephritis: Pathophysiology, treatment and future strategy. Clin J Am Soc Nephrol. 2011;6:679–89.

Feldt RH, Stickler GB. The gastrointestinal manifestations of anaphylactoid purpura in children. In: Proceedings of Staff Meetings of the Mayo Clinic, Rochester, Minnesota, vol 37. 1962. p. 465.

Gardner-Medwin JM, et al. Incidence of Henoch-Schönlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. Lancet. 2002;360(9341):1197.

Garzoni L, et al. Nervous system dysfunction in Henoch-Schonlein syndrome: systematic review of the literature. Rheumatology (Oxford). 2009;48(12):1524–9. Epub 2009 Sep 21.

Lau KK, et al. Serum levels of galactose-deficient IgA in children with IgA nephropathy and Henoch-Schönlein purpura. Pediatr Nephrol. 2007;22:2067.

Levy M, et al. Anaphylactoid purpura nephritis in childhood: natural history and immunopathology. Adv Nephrol Necker Hosp. 1976;6:183.

Narchi H. Risk of long term renal impairment and duration of follow up recommended for Henoch-Schönlein purpura with normal or minimal urinary findings: a systematic review. Arch Dis Child. 2005;90:916–20.

Ronkainen J, et al. The adult kidney 24 years after childhood Henoch-Schönlein purpura: a retrospective cohort study. Lancet. 2002;360(9334):666.

Saraclar Y, et al. Acute hemorrhagic edema of infancy (AHEI)–a variant of Henoch-Schonlein purpura or a distinct clinical entity? J Allergy Clin Immunol. 1990;86:473–83.

Saulsbury FT. Henoch-Schönlein purpura in children. Report of 100 patients and review of the literature. Medicine (Baltimore). 1999;78:395.

Saulsbury FT. Epidemiology of Henoch-Schonlein purpura. Cleve Clin J Med. 2002;69(Suppl 2): SII87–9.

Stewart M, et al. Long-term renal prognosis of Henoch-Schonlein purpura in an unselected childhood population. Eur J Pediatr. 1988;147:113–5.

Trapani S, et al. Henoch Schonlein purpura in childhood: epidemiological and clinical analysis of 150 cases over a 5-year period and review of literature. Semin Arthritis Rheumatol. 2005;35:143.

Trygstad CW, Stiehm ER. Elevated serum IgA globulin in anaphylactoid purpura. Pediatrics. 1971;47(6):1023.

Weiss PF, et al. Effects of corticosteroid on Henoch-Schonlein purpura: a systematic review. Pediatrics. 2007;120:1079–87.

Vasculitis: Kawasaki Disease

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Synonyms

Mucocutaneous lymph node syndrome

Definition

Kawasaki Disease (KD) is a childhood vasculitis affecting small- and medium-sized blood vessels and manifesting classically as fever, bilateral conjunctivitis, erythema of the lips and oral mucosa, cervical lymphadenopathy, rash, and extremity changes.

Introduction

Kawasaki Disease (KD) is the second most common childhood vasculitis and the leading cause of acquired heart disease among children in the

developed world (Burns and Glodé 2004). It is an acute, self-limited febrile illness of unknown cause, characterized by fever and signs of mucocutaneous inflammation. Fifteen to 25 % of untreated children develop coronary artery abnormalities, placing them at risk of developing ischemic heart disease or sudden death later in life (Kato et al. 1996). Therapy in the acute phase is aimed at reducing inflammation using intravenous immunoglobulin (IVIG), thereby minimizing the risk of coronary artery aneurysms and other late sequelae.

Diagnostic Criteria

Epidemiologic criteria for the diagnosis of KD remain largely unchanged since the initial description of the condition by Dr. Tomisaku Kawasaki in 1967 (Ayusawa et al. 2005):

Fever that persists for at least 5 days plus at least four of the following:

1. Bilateral nonsuppurative conjunctival injection
2. Changes of the lips and oral cavity
3. Cervical lymphadenopathy
4. Polymorphous exanthema
5. Changes in the peripheral extremities (such as erythema, swelling, or desquamation) or perineal area (an erythematous eruption)

Patients with at least 5 days of fever but less than four principal criteria can be diagnosed with KD on the basis of coronary artery abnormalities as seen on 2D echocardiography or angiography. The diagnosis may also be made in patients with just 4 days of fever possessing at least four of the principal criteria if the fever is alleviated by administration of IVIG.

As with all conditions diagnosed on the basis of clinical manifestations, sensitivity and specificity of the KD criteria are imperfect. Children who manifest five or six clinical criteria may have other conditions, including drug reactions, systemic onset juvenile idiopathic arthritis, or viral syndromes. One study of patients referred for possible KD found that the standard clinical diagnostic criteria for KD were fulfilled by 18 of 39 patients (46 %) who were later diagnosed with something else (Burns et al. 1991). Of greater concern is the fact that children may have an incomplete or atypical form of KD and remain at risk for developing coronary artery aneurysms despite not fulfilling diagnostic criteria. At least 10 % of those who develop coronary artery aneurysms never meet criteria for KD (Sundel 2002). This is actually not surprising in view of the fact that Dr. Kawasaki published his description of KD before cardiac involvement was recognized. To correct these deficiencies in the classical diagnostic criteria, an expert panel developed an algorithm which takes into account a child's risk of developing coronary artery changes based on laboratory and epidemiologic factors in addition to clinical findings (Newburger et al. 2004). Treating children using this algorithm, even though they may not meet epidemiologic criteria for the diagnosis of KD, results in a significantly decreased risk of coronary artery involvement (Yellen et al. 2010).

To summarize the algorithm:

1. Infants 6 months or younger who have had at least 7 days of fever and evidence of systemic inflammation without a clear explanation should have an echocardiogram performed.
2. Children who have the requisite 5 days of fever but only two or three clinical criteria should have laboratory investigations performed. If CRP is equal to or more than 3.0 mg/dL and/or ESR is equal to or greater than 40 mm/h, and they have at least three supplemental criteria (albumin $\leq$3.0 g/dL, anemia for age, elevation of alanine aminotransferase, platelets after 7 days $\geq$450,000/mm^3, white blood cell count $\geq$15,000/mm^3, and urine $\geq$10 white blood cells/high-power field), they should be treated and have an echocardiogram. Children who have less than three supplemental criteria may be treated for KD if the echocardiogram reveals findings suggestive of KD.

Pathogenesis

The etiology of KD is unknown but numerous epidemiologic features point to an unusual inflammatory response to one or more ubiquitous infectious pathogens in genetically predisposed individuals (Burgner and Harnden 2005).

Features in support of this mechanism include the preponderance of KD in children between 1 and 5 years of age (very young infants are presumably protected by passive maternal antibody transfer, while adults have already experienced asymptomatic infection) and its predominantly winter and springtime seasonality, coinciding with peaks in contagious disease incidence. In addition, KD demonstrates "epidemics," such as five- to tenfold spikes in incidence in Japan during the 1980s and 1990s. These spread centrifugally from incident communities, though cases of person-to-person transmission remained unusual, presumably because the prevalent infection only triggered KD in a small number of susceptible individuals. Nevertheless, extensive investigations using culture and serologic techniques as well as nucleic acid primers and deep sequencing have failed to identify consistent evidence of specific pathogenic trigger(s). This has led others to postulate alternative mechanisms for the development of KD, such as immune activation due to a superantigen, particularly considering the clinical and immunological similarities between KD and staphylococcal or streptococcal toxin-mediated illnesses (Matsubara et al. 2006). All such hypotheses remain merely theories, awaiting definitive confirmation.

At the cellular and molecular level, KD appears to be a T cell-mediated disease. Limited examination of autopsy specimens has shown infiltration of the coronary arterial wall by CD8+ T cells (Brown et al. 2001). During the acute illness, proinflammatory T effector memory cells are present in peripheral blood, while cessation of inflammation is associated with expansion of the regulatory T cell population (Franco et al. 2010). The pathways ultimately leading to vascular inflammation and damage are also incompletely elucidated, but it appears that endothelial cell activation and infiltration by CD68 monocyte/macrophages and oligoclonal IgA plasma cells leads to cytokine and matrix metalloproteinase-mediated arterial wall disruption.

The genetic basis of susceptibility is similarly uncertain. In some studies, certain polymorphisms of HLA have been linked to disease susceptibility and formation of coronary artery aneurysms. Genome-wide association studies have identified preferential expression in children with KD of five genes (LNX1, CAMK2D, ZFHX3, CSMD1, and TCP1) which are functionally related to inflammation, apoptosis, and cardiovascular pathology (Burgner et al. 2009), as well as ITPKC, which acts as a negative regulator of T cell activation (Onouchi et al. 2008). Additional studies have suggested an association of KD with a variety of other genes, including transforming growth factor-β and the Fc fragment of IgG low-affinity IIa receptor (FcϒR2A), among others. However, none of these associations accounts for more than a few percent of the incidence of KD. Further work, now ongoing, is needed to clarify the role of genetics in the development of KD.

Epidemiology

Kawasaki disease accounts for 23 % of all childhood vasculitides, making it the most common childhood vasculitis after Henoch-Schonlein purpura (Bowyer and Roettcher 1996). Ninety percent of cases occur in children younger than age 5 years, and boys are approximately 60 % more susceptible than girls. Distribution is worldwide with an Asian preponderance. Thus, the annual incidence of KD in the United Kingdom is 8 per 100,000 children under 5 years of age, and 20 per 100,000 children under 5 in the United States (Holman 2010). The rates in Taiwan and Japan are more than five times higher (Huang et al. 2009).

Clinical Manifestations

KD is a triphasic disease with an initial acute febrile phase lasting up to 14 days, a subacute phase of 2–4 weeks and a convalescent phase that can last months to years.

The *acute febrile phase* is characterized by persistent and high fever that is minimally responsive to antipyretics. The fever lasts an average of 12 days without treatment but may continue for weeks (Newburger et al. 2004). The remaining five cardinal features of KD tend to occur

sequentially, though the order in which they develop may vary from case to case.

Conjunctivitis affects 85 % of children and is generally bilateral, limbal sparing, nonsuppurative, and painless. Other ocular manifestations include anterior uveitis, keratitis, papilledema, vitreous opacities, and subconjunctival hemorrhages, often with accompanying photophobia.

Oral mucosal changes are seen in 90 % of affected children including red, dry, or cracked lips which may bleed, a "strawberry tongue," and diffuse injection of the oropharyngeal mucosa. Oral ulcers and pharyngeal exudates are rarely seen.

Cervical lymphadenopathy is the least common finding among the diagnostic criteria, affecting just 25 % of children with KD. When present, there tends to be a single enlarged lymph node limited to the anterior chain measuring more than 1.5 cm in diameter. The enlarged node is usually firm, non-fluctuant, and non-tender, without significant overlying erythema. An ill-defined cervical mass may prompt imaging with ultrasound or CT scan; this may demonstrate multiple enlarged nodes without suppuration, giving the appearance of a "bunch of grapes."

A *rash* is seen in 70–90 % of patients with KD and may take on many appearances. Most commonly it is macular, targetoid or morbilliform, and non-pruritic. Occasionally, it may appear urticarial, erythrodermic, or, rarely, pustular. Usually, the rash is extensive and involves the trunk and limbs, though in 2/3 of cases it begins in the perineal region where it eventually desquamates. Children who have had prior Bacille Calmette-Guerin (BCG) vaccination may have prominent erythema and induration of the BCG scar.

Extremity changes during the first week of KD take the form of erythema of the palms and soles and/or swelling of the dorsum of the hands and feet. During the third or fourth week, periungual desquamation – actual sheets of sloughed epidermis – is common.

Other clinical findings, while not included among the diagnostic criteria, may help distinguish KD from other febrile exanthems of childhood. The most noteworthy manifestations include irritability, anorexia, tachycardia out of proportion to the fever, arthralgias/arthritis (involving large joints during the acute phase of the illness, moving later to the small acral joints), diarrhea, vomiting, abdominal pain, cough, S3 gallop, muffled heart sounds, and rhinorrhea.

The *subacute phase* of KD generally begins towards the end of the second week of illness. It is characterized primarily by periungual desquamation of the fingers and toes, with possible extension to the palms and soles. Otherwise, children treated with IVIG are usually asymptomatic. Arthritis may persist into or develop during this period. Most of the coronary artery dilation and aneurysms that develop are detectable by this stage. Laboratory testing generally shows normalization of the CRP and white blood cell count, development of a reactive lymphocytosis, but an ESR that remains elevated for up to 6 weeks.

The *convalescent phase* is another period usually devoid of symptoms. By this time, normalization of inflammatory markers in the blood should be largely complete. One or two months after the onset of the fever, Beau's lines may appear. These are deep transverse grooves across the nails reflecting a nonspecific response to significant inflammation.

A more severe form of KD, with sustained hypotension or clinical signs of poor perfusion, has been described as Kawasaki shock syndrome. This variant is associated with more severe laboratory markers of inflammation, greater risk of coronary artery abnormalities, mitral regurgitation, and prolonged myocardial dysfunction. These patients may be resistant to immunoglobulin therapy and require additional anti-inflammatory treatment (Kanegaye et al. 2009).

Diagnosis and Evaluation

The diagnosis of KD is a clinical one, with no definitive diagnostic test available. Diagnosis is based on evidence of systemic and mucocutaneous inflammation, reflecting the diffuse medium and small vessel vasculitis underlying the disorder. As noted above, diagnostic criteria were supplemented in 2004 by an algorithm to facilitate identification and treatment of children failing to meet classical diagnostic criteria but who

are nonetheless at risk for developing coronary artery aneurysms (Newburger et al. 2004). Incomplete KD should be considered in all children with unexplained fever for over 5 days associated with two or three of the principal clinical features. Incomplete KD is more common in young infants, for whom the risk of developing coronary artery aneurysms is substantial. Laboratory findings are non-diagnostic but may heighten or reduce the suspicion in ambiguous cases. Children under 6 months of age with persistent fever for 7 days and evidence of inflammation should have an echocardiogram to screen for atypical KD even in the absence of clinical criteria. Supportive evidence includes perivascular brightness, ectasia, and lack of tapering of the coronary arteries. These findings may represent coronary arteritis before the formation of aneurysms, which are rarely apparent before day 10 of illness. Decreased left ventricular contractility, mild valvular regurgitation (most commonly mitral regurgitation), and pericardial effusion also may be seen.

Laboratory studies were not included in the diagnostic criteria for classic KD but they may support the diagnosis. The most consistent finding is evidence of systemic inflammation, as evidenced by elevated acute phase reactants. C-reactive protein (CRP) and neutrophilic leukocytosis are most characteristic. The erythrocyte sedimentation rate (ESR) is generally elevated as well, though in severe cases of KD complicated by a consumptive coagulopathy, the ESR may be normal or low. Thrombocytosis develops in most cases as well, though platelet counts generally do not rise above normal until the second week of illness. During the convalescent phase platelet counts may reach 1,000,000/mm^3.

Other laboratory findings may include a normocytic, normochromic anemia, sterile pyuria due to urethritis (and hence not evident on urine samples obtained via suprapubic aspiration or catheterization), and abnormal liver functions tests (due to intrahepatic congestion or obstructive jaundice from hydrops of the gall bladder). Hyponatremia, if present, is associated with an increased risk of coronary artery aneurysms. Other bodily fluids may reveal inflammation as well, including a mononuclear pleocytosis with depressed glucose and elevated protein levels in the cerebrospinal fluid, and joint fluid with up to 100,000 neutrophils per mm^3. Uveitis on slit lamp examination may provide further evidence for the diagnosis, since inflammation of the anterior chamber is seen more often in KD than in mimics of KD.

Differential Diagnosis

The clinical features of KD overlap with a variety of other childhood illnesses. These KD mimics include the following:

- Viral exanthems such as adenovirus, measles, enterovirus, echovirus, and EBV
- Less common infections such as Rocky Mountain Spotted Fever and leptospirosis
- Toxin-mediated illnesses including Staphylococcal scalded skin syndrome, toxic shock syndrome, and scarlet fever
- Drug reactions, particularly Steven-Johnson Syndrome
- Inflammatory conditions, most importantly systemic onset juvenile idiopathic arthritis and polyarteritis nodosa
- Hypersensitivity reactions, e.g., mercury toxicity

An additional challenge is the fact that up to 40 % of children with the diagnosis of KD simultaneously have evidence of a bacterial or viral infection. Documentation of such infections does not preclude the diagnosis of KD, though it may complicate evaluation of the patient. On the other hand, certain clinical features are preferentially seen in KD mimics compared to KD itself. Thus, a child is less likely to have KD if he has an exudative conjunctivitis, exudative pharyngitis, discreet intraoral lesions, bullous or vesicular rash, or generalized lymphadenopathy (Burns et al. 1991).

Management

The aims of therapy are to reduce inflammation and prevent development of coronary artery abnormalities. The American Heart Association describes standard of care as a single infusion of

intravenous immunoglobulin (IVIG 2 g/kg) and high-dose *aspirin* (80–100 mg/kg, maximum 4 g/day) within the first 10 days of illness. This therapy has been shown to reduce the incidence of coronary artery aneurysms from over 20 % to less than 5 % (Newburger et al. 1991). Once fever has been absent for 48 h, the aspirin dose may be reduced to 3–5 mg/kg daily until normalization of inflammatory markers or resolution of coronary aneurysms, if present. IVIG has the additional beneficial effect of normalizing serum lipoprotein profiles which otherwise may remain abnormal for years (Newburger et al. 2004).

IVIG is recommended before 10 days of disease but is also warranted after that time period in patients with clinical and laboratory evidence of ongoing inflammation and vasculitis. The benefits of IVIG in patients with established coronary artery aneurysms are more ambiguous, though the excellent tolerability of IVIG therapy favors its use whenever there is even a small chance that the patient will benefit.

Although numerous explanations of the efficacy of IVIG in KD have been proposed, the actual mechanism(s) remains uncertain (Kaveri 2012). IVIG contains a range of antibodies to microbial antigens and superantigens, cytokines, cytokine receptors, and endothelial antigens. Given early in the course of disease, IVIG ameliorates cytokine release, and prevents endothelial damage. Explanations of these effects include inhibition of monocyte/macrophage cytokine synthesis (especially TNF alpha and IL-1), blockade of cytotoxic reactions, and inhibition of complement activation. Meta-analyses suggest that aspirin does not add to the cardioprotective effects of IVIG, but it continues to form part of the recommended therapeutic regimen for KD because all significant studies of the effects of IVIG in KD included treatment with aspirin as well. Nonetheless, adverse effects of aspirin must be borne in mind, including raised liver enzymes and transient hearing loss. Gastrointestinal side effects are a theoretical risk but they are less common in children than adults. Reye Syndrome remains a concern and hence the drug should be discontinued if there is exposure to or signs of influenza or varicella infection.

Treatment of Refractory Disease

Approximately 10–15 % of patients fail to respond to initial treatment with IVIG. Failure is defined as persistence or recurrence of fever within 36 h from the end of the IVIG infusion. Fever prior to 36 h may be secondary to infusion reactions, but beyond this time systemic inflammation is likely due to KD. As such, it suggests ongoing vasculitis and is associated with an increased risk of coronary artery aneurysms. Current American Heart Association (AHA) guidelines recommend a second dose of IVIG in children who do not recover fully after initial treatment. If two or more doses of IVIG are ineffective in treating the fever and other signs of inflammation, pulse methylprednisolone (30 mg/kg daily for 1–3 doses) or infliximab (5 mg/kg) should be considered. Case reports have suggested that other agents, such as cyclosporine, ulinastatin, and cyclophosphamide, may also be beneficial in children with refractory KD. Additionally, when coronary artery aneurysms develop, antiplatelet therapy in the form of low-dose aspirin, and at times systemic anticoagulation with heparin or warfarin, may be needed to forestall arterial thromboses. Should ischemic symptoms or evidence of obstruction occur, additional antiplatelet or anticoagulant therapy and/or revascularization with thrombolytics or coronary angioplasty may be indicated. Such interventions should be left to tertiary care centers with considerable experience in treating complicated KD.

Vaccinations

Patients should not receive any vaccinations within 9–11 months of IVIG, with the exception of those who are exposed to an outbreak of any vaccine-preventable disease(s). Children above 6 months of age on chronic aspirin therapy should receive influenza and varicella immunization to minimize the risk of Reye Syndrome (American Academy of Pediatrics 2012).

Complications

Complications in KD primarily involve the cardiovascular system and are a consequence of

coronary artery thrombosis in inflamed aneurysmal vessels. In the acute phase, heart failure may occur, usually from myocardial inflammation rather than ischemia. IVIG often results in rapid clinical improvement. After the first week, new myocardial dysfunction is usually a result of ischemia or infarction. Electrocardiogram and echocardiography may be necessary to distinguish between inflammation and ischemia. Myocardial ischemia may also cause arrhythmias and this is usually the cause of the rare deaths seen in KD. Peripheral artery disease may result in ischemia or gangrene of the involved limb (Newburger et al. 2004).

Prognosis

The prognosis of KD is generally good in patients who receive IVIG (and appropriate salvage therapy if persistently febrile) during the acute phase of illness. Outcomes are primarily dependent on the presence or absence of coronary artery involvement and attendant risk for myocardial ischemia. The majority of patients recover without long-term cardiovascular sequelae. The reported mortality rate is 0.1–0.3 %, with death usually resulting from coronary artery thrombosis, myocardial infarction, or aneurysmal rupture. Mortality rates based on data from the Japanese registry of over 6,000 patients demonstrate an increased rate within the first 2 months of disease, which drops to population risk after the acute phase (Kato et al. 1996).

Morbidity is more difficult to measure. Children without cardiovascular abnormalities up to 8 weeks after onset of disease appear to be asymptomatic 10–21 years later but their longer-term atherosclerotic risk remains unknown. Coronary artery dilatation of <8 mm generally regresses over time and most smaller aneurysms (<6 mm in diameter) fully resolve by echocardiogram (Fukushige et al. 1996). About 20 % of patients who develop coronary artery aneurysms during the acute stage of the disease will develop stenosis. Patients with giant (8 mm or more) coronary artery aneurysms are at a long-term risk of developing aneurysm thrombosis or coronary artery stenosis and myocardial infarction even years after the acute illness (Kato et al. 1996).

Recurrence rates according to Japanese surveys have been recorded at 2 % of patients or 6.9 per 1,000 person-years, usually in younger children who had cardiac sequelae during the first episode, and within 12 months of the initial KD. The risk of cardiac sequelae is increased in patients with recurrent KD.

Follow-Up

Children with KD should be followed indefinitely regardless of echocardiographic findings. Although the long-term risk of developing coronary artery disease after acute KD is not known, late findings such as impaired endothelial function and carotid intimal thickening suggest that long-term vigilance is warranted (Newburger et al. 2004). The AHA and AAP have published guidelines for subsequent therapy, physical activity, and follow-up visits based on the risk for myocardial infarction (Newburger et al. 2004).

Cross-References

- ▶ Autoimmune Heart Disease: Animal Models
- ▶ Polyarteritis Nodosa
- ▶ Vasculitis: Henoch-Schönlein Purpura

References

American Academy of Pediatrics. In: Pickering LK, editor. Red Book: 2012 report of the committee on infectious diseases. 29th ed. Elk Grove Village: American Academy of Pediatrics; 2012. p. 37–8.

Ayusawa M, Sonobe T, Uemura S, et al. Revision of diagnostic guidelines for Kawasaki disease (the 5th revised edition). Pediatr Int. 2005;47:232.

Bowyer S, Roettcher P. Pediatric rheumatology clinic populations in the United States: results of a 3 year survey. Pediatric Rheumatology Database Research Group. J Rheumatol. 1996;23(11):1968–74.

Brown TJ, Crawford SE, Cornwall ML, Garcia F, Shulman ST, Rowley AH. CD8 T lymphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. J Infect Dis. 2001;184:940–3.

Burgner D, Harnden A. Kawasaki disease: what is the epidemiology telling us about the etiology? Int J Infect Dis. 2005;9:185.
Burgner D, Davila S, Breunis WB, et al. A genome-wide association study identifies novel and functionally related susceptibility loci for Kawasaki disease. PLoS Genet. 2009;5:e1000319.
Burns JC, Glodé MP. Kawasaki syndrome. Lancet. 2004;364:533.
Burns JC, Mason WH, Glode MP, et al. Clinical and epidemiologic characteristics of patients referred for evaluation of possible Kawasaki disease. United States Multicenter Kawasaki Disease Study Group. J Pediatr. 1991;118:680.
Franco A, Shimizu C, Tremoulet AH, Burns JC. Memory T-cells and characterization of peripheral T-cell clones in acute Kawasaki disease. Autoimmunity. 2010;43:317–24.
Fukushige J, Takahashi N, Ueda K, Hijii T, Igarashi H, Ohshima A. Long-term outcome of coronary abnormalities in patients after Kawasaki disease. Pediatr Cardiol. 1996;17(2):71.
Holman RC, Christensen KY, Belay ED, et al. Racial/ethnic differences in the incidence of kawasaki syndrome among children in Hawaii. Hawaii Med J. 2010;69(8):194-7.
Huang WC, Huang LM, Chang IS, et al. Epidemiologic features of Kawasaki disease in Taiwan, 2003–2006. Pediatrics. 2009;123:e401–5.
Kanegaye JT, Wilder MS, Molkara D, Frazer JR, Pancheri J, Tremoulet AH, Watson VE, Best BM, Burns JC. Recognition of a Kawasaki disease shock syndrome. Pediatrics. 2009;123(5):e783–9.
Kato H, Sugimura T, Akagi T, Sato N, Hashino K, Maeno Y, Kazue T, Eto G, Tamakawa R. Long-term consequestions of Kawasaki disease: a 10- to 21-year follow-up study of 594 patients. Circulation. 1996;94:1379–85.
Kaveri SV. Intravenous immunoglobulin: exploiting the potential of natural antibodies. Autoimmune Rev. 2012;11:792–4.
Matsubara K, Fukaya T, Miwa K, et al. Development of serum IgM antibodies against superantigens of Staphylococcus aureus and Streptococcus pyo-genes in Kawasaki disease. Clin Exp Immunol. 2006;143:427–34.
Newburger JW, Takahashi M, Beiser AS, et al. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. N Engl J Med. 1991;324:1633–9.
Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Pediatrics. 2004;114:1708–33.
Onouchi Y, Gunji T, Burns JC, Shimizu C, Newburger JW, Yashiro M, Nakamura Y, Yanagawa H, Wakui K, Fukushima Y, Kishi F, Hamamoto K, Terai M, Sato Y, Ouchi K, Saji T, Nariai A, Kaburagi Y, Yoshikawa T, Suzuki K, Tanaka T, Nagai T, Cho H, Fujino A, Sekine A, Nakamichi R, Tsunoda T, Kawasaki T, Nakamura Y, Hata A. ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. Nat Genet. 2008;40:35–42.
Sundel RP. Update on the treatment of Kawasaki disease in childhood. Curr Rheumatol Rep. 2002;4:474.
Yellen ES, Gauvreau K, Takahashi M, et al. Performance of 2004 American Heart Association recommendations for treatment of Kawasaki disease. Pediatrics. 2010;125:e234.

Viral Myocarditis

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Synonyms

Virus-mediated cardiomyopathy

Definition

In 1995, the World Health Organization assigned the following definition to inflammatory cardiomyopathy: "Inflammatory cardiomyopathy is defined by myocarditis in association with cardiac dysfunction. Myocarditis is an inflammatory disease of the myocardium and is diagnosed by established histological/immunological, and immunohistological criteria. Idiopathic, autoimmune, and infectious forms of inflammatory cardiomyopathy are recognized." Viral myocarditis sits in the latter portion of the WHO definition for inflammatory cardiomyopathy: an infectious form of the disease induced by a viral or combination of viral infections in the heart and leading to inflammation of the heart muscles. Virus-mediated cardiomyopathy includes direct virus-mediated damage to cardiomyocytes and associated secondary immune responses that ensue such as inflammation and autoimmunity.

Disease Incidence and Pathology

Assigning appropriate clinical symptoms to characterize viral myocarditis is difficult as the disorder presents a myriad of clinical forms ranging from nonspecific systemic symptoms to dilated cardiomyopathy to complete heart failure. The Dallas criteria were first established in 1984 to provide clinicians a set of pathological guidelines to follow with endomyocardial biopsies and for the diagnosis of myocarditis (Marchant et al. 2011). Diagnosis by endomyocardial biopsy allows an assessment of any viral presence and the extent of inflammation in the myocardium (Esfandiarei and McManus 2008; Yajima and Knowlton 2009).

Though the criteria were considered the gold standard for almost two decades, they didn't allow for identifying viral genomes in the heart. Evidence has accumulated in the past decade of many viruses linked to myocarditis, spurring the need for the Dallas criteria to be revisited. Following skeptical clinical trials using solely the Dallas criteria, diagnosis of myocarditis was supplemented with newly developed detection technologies such as polymerase chain reaction (PCR) to detect the presence of viral genomes, immunohistochemical staining for upregulated cardiac myocyte markers intercellular adhesion molecule (ICAM) and major histocompatibility complex class II (HLA-DR), and immunoserological testing for anticardiac myocyte antibodies. In situ hybridization and electron microscopy have also successfully identified viruses in myocarditis patients (Esfandiarei and McManus 2008; Yajima and Knowlton 2009).

Following viral infection, myocarditis patients may endure a phase of autoimmunity with viral and myosin epitope cross-reactivity. Depending on the severity of injury caused by both cellular and humoral autoimmunity, disease may progress to dilated cardiomyopathy (DCM). The potential onset of an autoimmune chronic disease demonstrates the importance of both host and viral genetics in the pathogenesis of viral myocarditis (Marín-García 2011).

Viruses Linked to Human Disease

A range of cardiotropic viruses have been linked to myocarditis using endomyocardial biopsy samples from patients with acute or dilated cardiomyopathy. Human cardiotropic viruses linked to myocarditis include adenoviruses, enteroviruses, parvovirus B19 (PVB19), human herpesvirus 6 (HHV6), cytomegalovirus, Epstein-Barr virus (EBV), influenza virus, hepatitis C virus (HCV), herpes simplex virus (HSV), and human immunodeficiency virus (HIV), where their genomes have been recovered using PCR and in situ hybridization techniques (Laurent 2011).

PVB19 and HHV6 have both been detected in patients with idiopathic DCM. Although the true etiology of viral myocarditis has yet to be established, the literature strongly implicated PVB19 as one of the most frequently detected cardiotropic viruses. Interestingly, PVB19 infects endothelial cells and avoids cardiac myocytes (Bock et al. 2010). PVB19 genomes dominate in isolated diastolic dysfunction unexplained cases. PVB19 likely contributes to endothelial dysfunction, increased inflammation, and the progression to diastolic dysfunction. In a recent follow-up study of 50 patients previously diagnosed with severe acute myocarditis, nested PCR detected mostly PVB19 genomes and, to a lesser extent, HHV6 in patients that had developed heart failure with a normal ejection fraction (HFNEF) in the long term (Eurlings and Heymans 2011). Clinical studies from Germany have also reported the extensive presence of PVB19 in myocarditis patients compared with other cardiotropic viruses. Still, it is important to acknowledge that PVB19 genomes have also been isolated from patients without myocarditis (Yajima 2011).

From a large-scale US study with myocarditis patients, a recurrent presence of adenoviruses and enteroviruses was detected using PCR on endomyocardial biopsy samples. Cytomegalovirus genomes were also identified though much less frequently than adenovirus genomes, which were predominant in both child and adult patients (Bowles et al. 2003; Yajima 2011). With human cytomegalovirus (HCMV), only mRNA for the

early viral enzymatic proteins can be detected in cardiomyocytes and fibroblasts since the herpesvirus likely establishes a latency phase, making the detection of mRNA for the late structural proteins difficult. The latency and reactivation mechanisms of herpesviruses like HCMV in heart tissue and their roles in myocarditis and the onset of chronic heart disease have yet to be understood.

Some groups suggest enteroviruses such as the Coxsackie group B serotypes (CVB) as the most likely agents responsible for myocarditis (Chapman and Kim 2008). Enteroviral RNA has been detected in both myocarditic and DCM patients though there is a range in percentages reported from various studies. The variability in reporting numbers is due to variability in detection techniques (Chapman and Kim 2008). Importantly, CVB serotype 3 provides stages of disease progression in mice models that mimic those seen in human myocarditis and DCM patients, urging the support and widespread use of this virus in animal model studies (Richer et al. 2007; Chapman and Kim 2008; Lincez et al. 2011).

The reduction in endocardial fibroelastosis cases following the instillment of the mumps vaccine suggested a potential role for the mumps virus in myocarditis-related pathogenesis. Human immunodeficiency virus (HIV) has also been linked to myocarditis. It has been suggested that cardiomyocytes become damaged and undergo apoptosis when HIV infection of immune cells induces pro-apoptotic ligands and signaling and the production of nitrous oxide. Respiratory tract viruses such as influenza A and Epstein-Barr have also been detected and associated with myocarditis cases. The pathogenic contribution of each virus individually or as a collective remains to be determined and will require the contribution of successful clinical and animal model studies using sufficiently large cohorts. Detection of viral genomes in the myocardium does not necessarily implicate the virus' pathogenicity or involvement in the disease. Recovered genomes or virus particles may be coincidental bystanders or secondary infection participants (Marín-García 2011).

Despite the range of viruses associated with human myocarditis, Coxsackievirus B3 is most commonly used in animal models to induce disease and to study the accompanying cellular and molecular mechanisms.

Experimental Models

CB3-Induced Myocarditis

Though rats, guinea pigs, and rabbits have been used to study virus-induced myocarditis, mice present as the most widely used and effective animal model system for studying the disease. Murine models allow for the separation of mechanisms underlying autoimmune myocarditis and viral myocarditis. Similar genetics with humans, cost-effective care, the availability of transgenic models, and susceptibility to a range of cardiotropic viruses are all advantages that allow mice to supersede other animal models for studying viral myocarditis (Lincez et al. 2011).

The cellular and molecular mechanisms underlying viral myocarditis are typically studied in animal models using Coxsackievirus B3 (CB3) though the entry mechanisms for other cardiotropic viruses such as encephalomyocarditis virus (EMCV) have been demonstrated. Though other virus-induced myocarditis models exist, the CB3 model is advantageous against other viruses. The virus strain, CB3, that induces myocarditis is conserved for human and mice. In fact, CB3 does not have a limited tropism for species. Also, CB3-induced myocarditis models can be divided into two separate models for studying either viral damage or viral-induced autoimmunity (Fairweather and Rose 2004).

Cardiotropic virus selection for either endothelial or myocyte cells in the myocardium has been observed for a range of viruses using an array of detection methods including, but not limited to, electron microscopy, in situ hybridization, immunohistochemistry, and PCR joint with laser capture microdissection. The mammalian viral receptor Coxsackie-adenoviral receptor (CAR) has been identified as the point of entry for CB3, EMCV, and other myocyte-targeting viruses. In fact, CAR-deficient mice infected with CB3 do not develop myocarditis. CB3 can also bind to and use decay accelerating factor (DAF) as

a receptor for endocytosis. After undergoing receptor-mediated endocytosis, the viruses release their genomes inside the myocyte where translation and replication to produce new productive virus progeny can ensue. CB3's RNA genome has a positive polarity, allowing it to be directly translated into a polyprotein upon release into the host cell. CB3's replication affects a vast distribution of host proteins and cellular pathways including death pathways that may contribute to the ultimate demise of infected cells in the myocardium (Marchant et al. 2011). Viral persistence is observed for a long course in murine models. Over this course, pathologies similar to those observed with human myocarditis such as necrosis, fibrosis, and calcification of the myocardium are observed in mice and have shown to lead to cardiac dilation similar to the development of DCM in humans.

The immunopathological phases observed with CB3-induced myocarditis in murine models include acute, subacute, and chronic phases. During the subacute or viremic phase, viral infection leads to myocyte lysis and the release of viral and cellular antigens that activate innate immunity responders such as macrophages and stimulates the production of cytokines IL-1 and IL-2 and the TNFs and IFNs (Lincez et al. 2011). This phase can last up to 4 days before merging into a subacute phase where NK cells are activated to release perforin and cytotoxic T cells, and B lymphocytes also activate and induce the production of neutralizing and antimyosin antibodies. The subacute phase ultimately leads to viral clearance up to day 14 postinfection. The last phase observed in CB3-induced myocarditis is the chronic stage of disease, where virus has been able to escape the initial immune defenses and persists in the myocardium (Chapman and Kim 2008; Yajima and Knowlton 2009; Lincez et al. 2011).

Experimental Autoimmune Myocarditis

As previously discussed, mice are critical experimental models for studying virus-induced myocarditis. To discern pathologic and physiological changes between acute infection and the autoimmune phase of disease, an experimental autoimmune myocarditis (EAM)-induced model was created. The EAM model pairs an inducer of myocarditis, an immunogen, and alike virus-induced myocarditis and mimics the chronic disease phase characteristically observed in susceptible mice challenged with virus like CB3. During EAM, stages of disease severity are tracked and given a score based on the level of inflammatory infiltrates observed at the peak of inflammation. Classic inducers of myocarditis in the EAM model include cardiac myosin with complete Freund's adjuvant and the pertussis toxin. An alternative to cardiac myosin is a myocardiogenic peptide derived from the alpha cardiac heavy chain that is emulsified in complete Freund's adjuvant. This peptide induces disease and peak inflammation in the heart by day 21 postinjection. A combination of self-antigen such as cardiac myosin and adjuvants injected in mice will induce the generation of cardiac myosin-specific autoantibodies and mimic the pathology observed in the hearts of mice challenged with CB3 at 3-week postinfection (Lincez et al. 2011). Another inducer used in EAM models is cardiac troponin I. Following injection in mice, troponin I causes an intense autoimmune response consisting of both humoral and cellular responses. Myocardial inflammation and fibrosis are observed with troponin I EAM models as demonstrated with programmed cell death-1 receptor deficient mice injected with troponin I that produce high-titered autoantibodies and develop cardiomyopathy. Interestingly, troponin I EAM models exude a genetic and sex bias in susceptibility to myocardial inflammation compared with other EAM models. The key components for studying myocarditis with an EAM model are the use of a defined immunogen from the same species as the one used as the model and to use genetically defined inbred susceptible strains for proper analysis of the cellular and molecular mechanisms driving autoimmune disease and resulting pathology in the heart. The choice of susceptible mice for EAM studies is critical as the host's complex genetics can influence the severity and resolution of an infection or challenge with immunogen and whether the host will succumb to an adverse autoimmune incidence or not. Cardiac myosin challenge will only induce disease in genetically

susceptible mice as demonstrated by Poffenberger et al. (Lincez et al. 2011).

Antigen-specific peripheral tolerance induction can be used in EAM models to determine underlying mechanisms of autoimmunity. Myosin-specific tolerization and tissue homogenates are both used as tools to prevent EAM and to study potential myocarditis-associated autoimmunity therapeutic intervention. In an effort to determine whether the induction of peripheral tolerance from a self-antigen could be used to prevent autoimmunity typically observed with CB3-induced myocarditis, Horwitz et al. used nonobese diabetic (NOD) mice in an EAM model. Tolerization to cardiac myosin with a covalently coupled antigen approach before CB3 infection did not prevent autoimmune disease onset and demonstrated the ineffectiveness of targeting a single heart antigen for the prevention of viral-mediated autoimmune myocarditis (Richer and Horwitz 2009; Nussinovitch and Shoenfeld 2011). The application of an EAM model helped reveal the possibility of other cardiac antigens and not solely cardiac myosin as the autoantigen responsible for the onset of autoimmunity in viral-mediated myocarditis.

EAM is a critical tool for studying the cellular and molecular mechanisms underlying viral myocarditis and associated autoimmunity. Though the EAM model is rather artificial, it has implicated many key immune players contributing to disease pathogenesis and autoimmune mechanisms in vivo in the absence of an infective agent.

Immunomodulation

Yajima et al. discuss four separate phases from the perspective of the virus that define viral myocarditis: the preinfection phase where susceptibility factors and prevention from the onset of disease should be considered, phase 1 encompassing the time from initial virus infection in the myocardium through viral replication and lasting until viral replication ends, phase 2 when viral replication is undetectable though viral genomes persist, and phase 3 when viral genomes are undetected and patients are treated for ailing chronic heart conditions such as DCM (Yajima and Knowlton 2009). During Yajima et al.'s proposed phase 1 and following viral entry into cardiomyocytes or endothelial cells, many key immune players contribute to the elimination of virus and the initiation of inflammatory and possible autoimmune responses. If the innate response is incapable in controlling and eliminating viral replication, chronic disease in the myocardium can materialize and lead to the onset of debilitating conditions like DCM and heart failure. The key innate immune players include the toll-like receptors (TLRs), the double-stranded viral RNA sensors retinoic acid-inducible protein I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5), Janus kinase (JAK) and signal transducers and activators of transcription (STAT) signaling, and cytokines such as the interferons (IFN)-α/β, γ, interleukin IL-6, IL-1β, IL-4, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β (Lincez et al. 2011; Marín-García 2011).

Viruses harboring double-stranded RNA are sensed by TLR-3, MDA-5, and RIG-I. Activation of these viral sensors and associated signaling pathways is suggested to induce type I IFN responses. TLR3 knockout mice are impaired in controlling CB3-mediated myocarditis. Mice deficient in the viral sensor have enhanced disease and reduced IFN production and other key modulators of inflammation such as IL-1β and IL-12 following CB3 infection. TLR2, TLR4, and TLR8 are other pathogen recognition receptors implicated in viral myocarditis pathogenesis. TLR2 and TLR8 can be activated by cardiac myosin epitopes to induce an inflammatory state in the myocardium, and mice deficient in TLR4 reduces viral pathogenesis and the production of IL-1β and IL-18. Knocking out myeloid differentiation primary response gene (MyD88) in mice also protects mice from enhanced disease progression in both CB3-induced and EAM models (Hofmann et al. 2011; Lincez et al. 2011; Marín-García 2011).

Antigen-presenting cells (APCs) also play a critical role in disease progression. APCs influence lymphocyte responses. With two signals, antigen-loaded MHC and T cell receptor engagement and costimulatory signals CD40

and B7, APCs activate T cells and regulate helper T cell responses, peripheral tolerance, and regulatory T cells responses. Macrophages and dendritic cells (DCs), specialized APCs, produce cytokines and chemokines once activated, influencing the surrounding immunoenvironment and immune responses that affect disease pathogenesis. DCs in CB3 infected mice are defective in cytokine and chemokine production, impairing their ability to maintain peripheral tolerance and controlling the onset of autoimmune myocarditis. Macrophages and the immunomodulator TGF-β are critical in maintaining a balance between antiviral responses and avoidance of autoimmunity in CB3-induced myocarditis. It is also interesting to note the difference in macrophage phenotype for male and female mice in a CB3-induced myocarditis model. Male mice are typically most susceptible and demonstrate severe disease with CB3 infection compared to females. It has been suggested that this may be the influence of protective myocardial infiltrating macrophages observed in female mice. A gender bias in the cytokine signature infiltrating the myocardium has also been demonstrated with virus-induced myocarditis. Cytokines and chemokines that influence disease pathogenesis in EAM and virus-induced models include IL-2, IL-4, IL-6, IL-10, IL-12, IL-17, IL-23, TGF-β, IFNs, CCL2, and CXCL10 (Lincez et al. 2011).

Therapeutic Approaches, Challenges, and Future Directions

Generally, myocarditis treatment focuses on treating symptoms of heart dysfunction. If initial treatments fail and disease progresses to DCM, the remaining rescue treatment is heart transplantation. Unfortunately, in an effort to target viruses likely contributing to disease pathogenesis, broad-spectrum antivirals have been tested in trials with children and adults with little to none success. However, patients that have undergone endomyocardial biopsies with confirmation of enteroviral infection have been treated with IFNs-α/β and show significant clinical improvements including a reduction in inflammation and virus persistence in the heart.

Since there is a range in possible etiological agents responsible for viral myocarditis, it is difficult to design specific therapeutic interventions (Schultheiss et al. 2011). Nonetheless, the use of animal models and tools, such as the EAM and transgenic models, has identified key host and viral components that may present as successful therapeutic targets. The CB3 proteases 2A and 3C have been identified as contributors to the direct destruction of cardiac myocytes via activation of the caspase-8-mediated pathway. Inhibitors targeting these viral proteases may present as effective anti-CB3 and myocarditis therapeutic agents. Antiviral approaches are however risky, and the susceptibility to viral evasion and development of resistance must be considered. Another challenge in targeting viruses linked to myocarditis is the lack of understanding in the molecular mechanisms that influence reactivation of latent viruses such as PVB19 and the activation of immune pathways that instigate the reemergence of viral replication and the onset of chronic disease in the myocardium.

Cross-References

- ▶ Autoimmune Heart Disease: Animal Models
- ▶ Autoimmune Myocarditis and Pericarditis
- ▶ Interleukin-6
- ▶ TGF-β
- ▶ Tregs in the Liver
- ▶ Viral Myocarditis

References

Bock CT, Klingel K, et al. Human parvovirus B19-associated myocarditis. N Engl J Med. 2010;362(13):1248–9.

Bowles NE, Ni J, et al. Detection of viruses in myocardial tissues by polymerase chain reaction. Evidence of adenovirus as a common cause of myocarditis in children and adults. J Am Coll Cardiol. 2003;42(3):466–72.

Chapman NM, Kim KS. Persistent coxsackievirus infection: enterovirus persistence in chronic myocarditis and dilated cardiomyopathy. Curr Top Microbiol Immunol. 2008;323:275–92.

Esfandiarei M, McManus BM. Molecular biology and pathogenesis of viral myocarditis. Ann Rev Pathol: Mech Dis. 2008;3(1):127–55.
Eurlings C, Heymans S. Diastolic heart failure after acute myocarditis, a new discovery in inflammatory cardiomyopathies? Heart. 2011;97(9):685–6.
Fairweather D, Rose NR. Models of coxsackievirus-B3-induced myocarditis: recent advances. Drug Discov Today: Dis Models. 2004;1(4):381–6.
Hofmann U, Ertl G, et al. Toll-like receptors as potential therapeutic targets in cardiac dysfunction. Expert Opin Ther Targets. 2011;15(6):753–65.
Laurent A. Viral Myocarditis: physiopathology and diagnosis. In: Cihakova D, editor. Myocarditis. New York: InTech; 2011. p. 87–104.
Lincez PJ, Walic M, et al. The key players in coxsackievirus-induced myocarditis. In: Cihakova D, editor. Myocarditis. New York: InTech; 2011.
Marchant D, Garmaroudi FS, et al. Cellular and immunological regulation of viral myocarditis. In: Cihakova D, editor. Myocarditis. New York: InTech; 2011. p. 269–92.
Marín-García J. Signaling in endomyocarditis. Signaling in the heart. New York: Springer; 2011. p. 247–56.
Nussinovitch U, Shoenfeld Y. The clinical and diagnostic significance of anti-myosin autoantibodies in cardiac disease. Clin Rev Allergy Immunol. 2011;44:98–108.
Richer MJ, Horwitz MS. Preventing viral-induced type 1 diabetes. Ann N Y Acad Sci. 2009;1173:487–92.
Richer MJ, Poffenberger MC, et al. Early inflammatory responses direct chronic autoimmunity development in the heart following coxsackievirus infection. Future Virol. 2007;2(3):283–91.
Schultheiss HP, Kuhl U, et al. The management of myocarditis. Eur Heart J. 2011;32(21):2616–25.
Yajima T. Viral myocarditis: potential defense mechanisms within the cardiomyocyte against virus infection. Future Microbiol. 2011;6(5):551–66.
Yajima T, Knowlton KU. Viral myocarditis: from the perspective of the virus. Circulation. 2009;119(19):2615–24.

Vitiligo

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Synonyms

Leukoderma

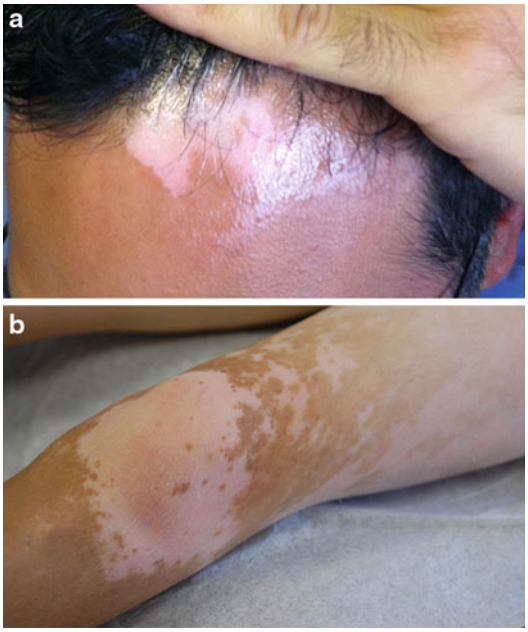
Definition

Vitiligo connotes a group of acquired disorders that result from loss of melanocytes, the pigment-producing cells of the body (Fig. 1a). Melanocytes are located primarily in the skin, hair, and eyes and to a lesser extent in the mucosal tissues, ears, heart, lungs, and adipose tissue. Melanocytes of the skin, hair, and mucosa are the targets in vitiligo, although in rare instances, melanocytes in the eyes and ears are also destroyed. The most common sites for vitiligo lesions are periorificial areas such as around the eyes, mouth, and genitalia and overlying joints. Lesions can progress, stabilize, or repigment. Repigmentation can occur after years of inactivity. Vitiligo affects both males and females at the same rate with 1–2 % of people afflicted worldwide, although in some areas, rates can reach 8 %. Disease onset can occur at any age but generally occurs in the first two decades of life.

Vitiligo has a significant impact on the physical and mental health of affected individuals, as well as on their quality of life. Lack of pigmentation compromises photoprotection of the skin, which is then more susceptible to solar damage. Melanocytes in the eyes are occasionally lost causing photophobia and night blindness. However, the most severe impact of vitiligo is on psychological well-being. Vitiligo frequently affects visible areas such as the face and hands causing additional distress, particularly among individuals with darker skin pigmentation (Nordlund 1992).

Diagnosis of vitiligo is generally based on clinical features including history, presence of depigmentation (rather than hypopigmentation), distribution, as well as (if present) evidence of repigmentation. The use of a Wood's lamp may be helpful when examining light-skinned individuals. Unlike disorders of melanocyte migration like piebaldism and Waardenburg syndrome where patches devoid of melanocytes are typically present from birth, lesions of vitiligo are generally acquired and progress over time. The location of lesions is also different.

Various forms of vitiligo, defined by the distribution of lesions, have been identified. Generalized vitiligo, also known as vitiligo vulgaris,



Vitiligo, Fig. 1 (**a**) Depigmented scalp lesion. (**b**) Depigmentation of the knee with evidence of follicular melanocyte repigmentation (Source: S. J. Orlow MD PhD)

is the most common form with widely distributed and symmetric progressive lesions. By contrast, segmental vitiligo presents with unilateral depigmented patches that are quasi-dermatomal. Segmental vitiligo has an early onset and a more limited and predictable course than generalized vitiligo. It does not generally progress beyond the single body segment affected. In contradistinction to segmental vitiligo, generalized vitiligo is associated with an increased incidence of certain other autoimmune disorders, including rheumatoid arthritis and diabetes (Alikhan et al. 2011). The focus of this entry is on generalized vitiligo, which is referred to as vitiligo hereafter.

Etiology of Vitiligo

Vitiligo is a multifactorial, polygenic disorder, and the precise molecular mechanisms underlying disease etiology are poorly understood. Depigmented lesions are devoid of melanocytes; however, precisely how melanocyte loss occurs remains to be elucidated. Susceptibility to vitiligo is genetically determined. Affected individuals may have a family history of vitiligo, while genetic association studies have been performed that identified disease susceptibility genes. Concordance among siblings is 6 % (Alkhateeb et al. 2003), although the majority of cases are sporadic. It is believed that environmental exposure to a trigger then determines if a susceptible individual will actually develop the disease.

In many cases, the trigger event is not known, hence the classification of most cases as idiopathic vitiligo. In some cases, vitiligo is known to occur due to exposure to a chemotoxin, resulting in contact vitiligo. When exposure occurs in the workplace, it is classified as occupational vitiligo. Despite the varying nature of the triggers, the same mechanisms are thought to underlie disease onset and progression, with no other discerning features separating the various forms. Thus, a number of recent studies aimed at elucidating the pathobiology of vitiligo have used contact vitiligo as a model.

Oxidative stress is thought to be a key factor in the early stages of vitiligo, prior to the onset of autoimmune disease. Studies have demonstrated increased markers of oxidative stress in serum and lesional skin from individuals with vitiligo, including increased expression of antioxidants such as superoxide dismutase and catalase. Genetic association studies in some population groups have shown linkage between antioxidant response genes and increased risk of developing vitiligo.

A neuronal component to vitiligo etiology has also been suspected. Melanocytes share neural crest origins with nerves, while lesions of vitiligo are sometimes more common at nerve endings. It has been suggested that neuromediators are released leading to melanocyte death (Le Poole et al. 1993).

While there is typically no overt inflammation at lesion sites (e.g., redness is lacking), there is an increase of proinflammatory cytokines in perilesional skin. Thus, it has been suggested that "microinflammation" precedes autoimmunity in vitiligo (Taieb 2012). Further evidence of inflammation is the presence of infiltrating CD4+ and CD8+ T cells at the periphery of lesions.

Although melanocytes are the target in vitiligo, the disease may be a reflection of keratinocyte dysfunction. Keratinocytes produces growth factors and hormonal signals that elicit survival responses in the melanocyte, for example, exposure to ultraviolet light (UV) results in the production of α-melanocyte-stimulating hormone, a ligand for the melanocortin-1 receptor (MC1R) on the surface of the melanocyte. MC1R activation promotes melanin synthesis but also stimulates pro-survival pathways such as DNA repair functions (Kadekaro et al. 2003).

Cell adhesion dysregulation is also thought to contribute to loss of melanocytes in vitiligo. A recent study showed an association between discoidin domain receptor 1 (DDR1) and vitiligo susceptibility. DDR1 is a collage-activated transmembrane tyrosine kinase receptor that facilitates E-cadherin adhesion to collagen type IV (Silva de Castro et al. 2010).

It is possible that several factors combine to trigger vitiligo. It has been proposed that a trigger event causes melanocyte stress, most likely in the form of oxidative stress. Melanocytes of individuals susceptible to developing vitiligo are unable to effectively mount an antioxidant response causing cell damage that leads to apoptosis and/or increased antigen presentation. A subsequent autoimmune response then perpetuates melanocyte death resulting in the progressive appearance and broadening of depigmented patches.

Generalized Vitiligo Is an Autoimmune Disorder

While the initial steps may involve oxidative damage, by the time of presentation, vitiligo appears to be primarily an autoimmune disorder. Melanocytes in lesional areas are presumed to be targeted for immune destruction. Antibodies against a number of melanocyte-specific proteins have been identified in sera from patients with vitiligo. Furthermore, individuals with vitiligo sometimes present with other autoimmune disorders that affect melanocytes (halo nevi), the skin (psoriasis), and other organs including Addison's disease, adult-onset type 1 diabetes mellitus, rheumatoid arthritis, Graves' disease, and systemic lupus erythematosus.

The most significant progress in understanding the immune component in vitiligo progression stems from genetic association studies that have identified key genes that contribute to increased susceptibility to vitiligo (Jin et al. 2012; Spritz 2012). Some of these genes play a role in immunity, while others encode melanocyte proteins that serve as antigens and elicit the autoimmune response (Table 1). Interestingly, some genes that are associated with increased risk of melanoma are associated with a lower risk of vitiligo. It has been suggested that these genes contribute to the ability of cells to evade immune detection in melanoma, while causing melanocytes to be targeted more easily by the autoimmunity in vitiligo. A prime example of this may be the association of a variant of the melanocyte-specific protein tyrosinase.

Tyrosinase is an enzyme that is critical for the biosynthesis of melanin. It undergoes extensive posttranslation modification to ensure functionality. A polymorphism at amino acid 402, among Caucasians, encodes for either glutamine (402Q) or more commonly arginine (R402). The 402Q variant is hypoglycosylated and is not readily processed for antigen presentation, thus decreasing the risk of vitiligo but increasing melanoma risk (Spritz 2012). A number of antibodies targeting melanocyte-specific proteins have been identified in addition to tyrosinase, including tyrosinase-related proteins 1 and 2, Pmel17, and MC1R.

It should be noted that there are some differences in the genes associated with vitiligo in different population groups; thus, a cohesive overall picture of vitiligo pathobiology is likely to consist of multiple pathways that contribute to varying degrees in disease onset and progression.

Immune Response in Vitiligo

Inflammatory cells are present in perilesional skin, and T cells harvested from perilesional areas can target melanocytes (van den Boorn et al. 2009). Although CD8+ and CD4+ T cells

Vitiligo, Table 1 A subset of genes associated with increased risk of vitiligo and other autoimmune disorders or with vitiligo alone

Gene class	Gene	Associated disorders
Plasma cell differentiation	BACH2 basic leucine zipper transcription factor 2	GD
Member of C1qTNF family may be involved in innate immunity	C1QTNF6 C1q and tumor necrosis factor-related protein 6	T1D, RA
Apoptosis	CASP7 apoptosis-related cysteine peptidase 7	
B-cell differentiation and maturation	CCR6 chemokine (C-C motif) receptor 6	IBD, RA, GD
Cell-cell and cell-matrix interactions	CD44	T2D
T-cell proliferation and cytokine production	CD80	Juvenile idiopathic arthritis
Immunoreceptor signaling	CLNK cytokine-dependent hematopoietic cell linker	ALS
T-cell regulation	CTLA-4 cytotoxic T-lymphocyte-associated protein 4	T1D, GD, IBD
MHC class II	HLA-DQA1 major histocompatibility complex, class II, DQ alpha 1	Multiple including GD and arthritis juvenile rheumatoid
MHC class II	HLA-DRB1 major histocompatibility complex, class II, DR beta 1	RA, IDD, AID, SLE
B-cell differentiation	FOXP1 forkhead box P1	
T-cell regulation	FOXP3 forkhead box P3	? T1D
Induction of apoptosis by cytotoxic T cells	GZMB granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	Stevens-Johnson syndrome
	HCG9 HLA complex group 9 (non-protein coding)	
MHC class I	HLA-A, major histocompatibility complex, class I, *A*	AH
MHC class I	HLA-G, major histocompatibility complex, class I, *G*	RA, T1D, SLE
MHC class I	HLA-J, major histocompatibility complex, class I, *J* (pseudogene)	
Innate immunity	IFIH1, interferon induced with helicase C domain 1	(Melanoma)
	IKZF4, IKAROS family zinc finger 4 (Eos)	AA
Immune modulation	IL2RA, interleukin 2 receptor alpha	RA
Cell adhesion	LPP, LIM domain containing preferred translocation partner in lipoma	CD, RA
Melanocyte function	MC1R, melanocortin 1 receptor (alpha melanocyte-stimulating hormone receptor)	(Melanoma)
Apoptosis/inflammation	NLRP1, NLR family, pyrin domain containing 1	Multiple disorders including autoimmune diabetes, rheumatoid arthritis, and psoriasis
Melanocyte function	OCA2, oculocutaneous albinism II	(Melanoma)
T-cell receptor signaling	PTPN22, protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	GD, HT, RA, SLE
Apoptosis related	RERE, arginine-glutamic acid dipeptide (RE) repeats	(Linked to antioxidant regulator Nrf2)

(*continued*)

Vitiligo, Table 1 (continued)

Gene class	Gene	Associated disorders
T-cell receptor activation	SH2B3, SH2B adaptor protein 3	IDD, CD
Negatively regulates T-cell receptor signaling	SLA, Src-like adaptor	(Melanoma?)
Innate immunity	TICAM1, toll-like receptor adaptor molecule 1	(Melanoma)
Release of T-cell-attracting chemokines	TSLP	
Melanocyte function	TYR, tyrosinase	(Melanoma)
Regulates T-cell receptor signaling	UBASH3A, ubiquitin associated and SH3 domain containing A	T1D
Differentiation of plasma cells/stress response	XBP1, X-box binding protein 1	CRD

Compiled from data provided at http://www.genecards.org/ (Spritz 2012) and (Jin et al. 2012)
Abbreviations: *AA* Alopecia areata, *ALS* Amyotrophic lateral sclerosis, *AH* Autoimmune hepatitis, *AID* Autoimmune diabetes, *CD* Celiac disease, *CRD* Crohn's disease, *GD* Graves disease, *HT* Hashimoto's thyroiditis, *IBD* Inflammatory bowel disease, *IDD* Insulin-dependent diabetes, *RA* Rheumatoid arthritis, *SLE* Systemic lupus erythematosus, *T1D* Type 1 diabetes

are both present, there is a greater presence of CD8+ cells. CD8+ cells have been implicated in other autoimmune disorders such as diabetes. CD4+ cells are thought to facilitate the expansion of CD8+ cells prior to migration to the skin. CD8+ cells are activated when CTLA-4, encoded by a gene that is also associated with risk of developing vitiligo, is knocked out, but only if CD4+ cells are present. A recently developed mouse model for vitiligo involved the transplant of CD8+ T cells that target melanocytes in mice engineered to support interfollicular melanocytes. Depigmentation required additional treatment with interferon-γ (Harris et al. 2012). In a chicken model of vitiligo, interferon-γ expression was increased as melanocyte loss began (Shi and Erf 2012). In addition to interferon-γ, other cytokines that may facilitate an autoimmune response are also elevated, including interleukin 6 (IL-6) and IL-17. Increased IL-6 and IL-17 expression may account for the increase in Th17 cells at the periphery of active lesions. Langerhans cells are also present and may facilitate the activation of the Th17 cells (Wang et al. 2011).

While CD4+/CD8+ cell numbers increase, there is a concomitant reduction in the number of regulatory T cells (Tregs), which may also contribute to vitiligo pathogenesis. Tregs are immunosuppressants and reduced the risk of developing autoimmunity against "self" proteins. Foxp3, a marker for Tregs, has been shown to be associated with vitiligo (Spritz 2012), while expression of the skin homing chemokine CCL22 is reduced in perilesional skin (Klarquist et al. 2010). Thus, multiple mechanisms may contribute to the reduced presence of Tregs.

Linking Events That Trigger Vitiligo with Autoimmunity

While oxidative stress plays a role in triggering vitiligo and autoimmunity plays a role in disease progression, the link between these events is not well understood. It was recently demonstrated that chemical agents that trigger vitiligo, such as phenolics, induce oxidative stress that disrupts the protein folding machinery of the endoplasmic reticulum (ER). The redox balance of the ER is carefully regulated given that protein folding, disulfide bond formation, and recycling of folding chaperones require oxidation-reduction reactions. In perilesional skin and melanocyte from individuals with vitiligo, the ER is dilated suggested that homeostasis is indeed induced. ER dysregulation leads to the activation of a stress response known as the unfolded protein

response (UPR). UPR activation following exposure to phenolics results in activation of the UPR and subsequent expression of cytokines IL-6 and IL-8 (Toosi et al. 2012) that may lead to recruitment of immune components to the skin. In combination with the processing and presentation of melanocyte antigens, this recruitment may lead to autoimmunity in susceptible individuals.

Treatment Strategies for Vitiligo

There are few predictors of disease progression. While some individuals experience extensive pigment loss that eventually affects the entire body, others experience episodic changes in lesion size and number. Depigmentation continues long after exposure to the triggering agent, while spontaneous repigmentation can occur by migration of melanocytes from perilesional skin or unaffected hair follicles into areas of epidermal melanocyte loss. Current treatments include:

- Corticosteroids, which are thought to act through modulation of the immune system. They are frequently the first line of treatment and can be used in adjuvant therapy. Although typically used as topical agents, systemic corticosteroids are sometimes employed when spread is rapid.
- Topical calcineurin inhibitors such as tacrolimus which inhibit certain T-cell functions and reduce cutaneous inflammation, but lack some of the side effects of corticosteroids, have also been shown to be effective.
- Phototherapy is often utilized. Ultraviolet B (UVB) especially narrowband UVB (311 nm) is the most widely utilized form. Psoralen in combination with UVA (PUVA) is also effective but has fallen out of favor due to the increased risk of skin cancer (especially nonmelanoma skin cancer) and persistent photosensitivity. Excimer laser therapy (essentially a targeted UVB treatment) has been gaining popularity, with demonstrable improvement in 95 % of patients in some studies.
- Surgical treatment is considered an option in the treatment of recalcitrant vitiligo. Options include suction blister grafting, punch grafting, or grafting of melanocyte suspensions. Surgical options increase the risk of scarring and patchy repigmentation.
- Depigmentation therapy is useful in patients with extensive lesions where there is no longer hope of repigmentation. Monobenzyl ether of hydroquinone (a phenolic that can itself provoke vitiligo) is approved for this purpose; however, depigmentation is not always complete.

Numerous additional therapies have been suggested or tested for efficacy in vitiligo. Certain plant-derived substances such as *Ginkgo biloba* and *Polypodium leucotomos* extract have shown early promise in augmenting the effectiveness of UVB treatment when administered orally. No therapy is effective in all patients and long-term success rates are variable. Furthermore, many are accompanied by potential side effects including skin atrophy, hyperpigmentation, and scarring (Whitton et al. 2010).

There is no current protocol for evaluating which patients would benefit from a particular treatment or which patients are refractory to particular treatments. It is however clear that in order for agents that work by promoting melanocyte migration into depigmented areas to be effective, the hairs in these areas must remain pigmented so that there is a reservoir of follicular melanocytes. The success of a therapy is often heralded by formation of pigmented circles around hair follicles within the lesion as melanocytes migrate up and out of the follicles (Fig. 1b).

Recent suggestions for therapeutics include inhibitors of tumor necrosis factor-α (TNF-α) since studies have shown increased levels of TNF-α in perilesional skin. An additional therapeutic target may be IL-17A, which has also been shown to be elevated in perilesional skin (Wang et al. 2011).

Development of therapies has been hampered by the lack of clarity with regard to the pathobiology of vitiligo. With greater lucidity on the precise immune mechanisms that result in persistent melanocyte loss, it may be possible to develop more targeted therapies. It should however be noted that genetic association studies

suggest that melanocyte targeting by the immune system perpetuates melanocyte loss but is also crucial for immune surveillance that prevents melanoma.

Cross-References

- Antioxidants
- Chemokines
- CTLA-4
- Cytotoxic T Lymphocytes
- Interleukin-6
- PTPN22
- T Cell Memory
- Therapeutic Considerations in Kidney Diseases Due to Glomerulonephritis
- Tregs in the Liver

References

Alikhan A, Felsten LM, et al. Vitiligo: a comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. J Am Acad Dermatol. 2011;65(3):473–91.

Alkhateeb A, Fain PR, et al. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. Pigment Cell Res. 2003;16(3):208–14.

Harris JE, Harris TH, et al. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-gamma for autoreactive CD8(+) T-cell accumulation in the skin. J Invest Dermatol. 2012;132(7):1869–76.

Jin Y, Birlea SA, et al. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. Nat Genet. 2012;44:676–80.

Kadekaro AL, Kavanagh RJ, et al. Cutaneous photobiology. The melanocyte vs. the Sun: who will win the final round? Pigment Cell Res. 2003;16(5):434–47.

Klarquist J, Denman CJ, et al. Reduced skin homing by functional Treg in vitiligo. Pigment Cell Melanoma Res. 2010;23(2):276–86.

Le Poole IC, Das PK, et al. Review of the etiopathomechanism of vitiligo: a convergence theory. Exp Dermatol. 1993;2(4):145–53.

Nordlund JJ. The pigmentary system and inflammation. Pigment Cell Res. 1992;5:362–5.

Shi F, Erf GF. IFN-gamma, IL-21, and IL-10 Co-expression in evolving autoimmune Vitiligo lesions of smyth line chickens. J Invest Dermatol. 2012;132(3 Pt 1):642–9.

Silva de Castro CC, do Nascimento LM, et al. Genetic variants of the DDR1 gene are associated with vitiligo in two independent Brazilian population samples. J Invest Dermatol. 2010;130(7):1813–8.

Spritz RA. Six decades of vitiligo genetics: genome-wide studies provide insights into autoimmune pathogenesis. J Invest Dermatol. 2012;132(2):268–73.

Taieb A. Vitiligo as an inflammatory skin disorder: a therapeutic perspective. Pigment Cell Melanoma Res. 2012;25(1):9–13.

Toosi S, Orlow SJ, Manga P. Vitiligo-inducing phenols activate the unfolded protein response in melanocytes resulting in upregulation of IL6 and IL8. J Invest Dermatol. 2012;132(11):2601–9.

van den Boorn JG, Konijnenberg D, et al. Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. J Invest Dermatol. 2009;129(9):2220–32.

Wang CQ, Cruz-Inigo AE, et al. Th17 cells and activated dendritic cells are increased in vitiligo lesions. PLoS One. 2011;6(4):e18907.

Whitton ME, Pinart M et al. Interventions for vitiligo. Cochrane Database Syst Rev(1): CD003263; 2010.

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