

Current Directions in Autoimmunity

Series Editor:

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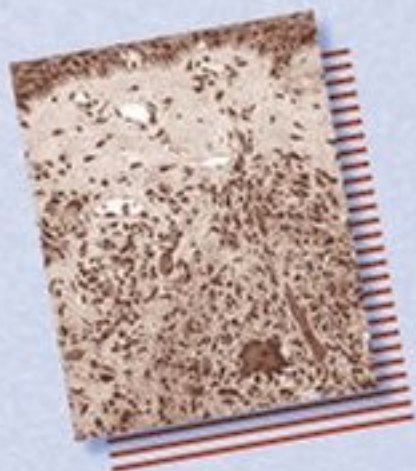
Vol. 10

Dermatologic Immunity

Editors

B.J. Nickoloff

F.O. Nestle



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A.N. Theofilopoulos La Jolla, Calif.

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Dermatologic Immunity

Volume Editors

Brian J. Nickoloff Chicago, Ill.

Frank O. Nestle London

45 figures, 4 in color, and 25 tables, 2008

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Library of Congress Cataloging-in-Publication Data

Dermatologic immunity / volume editors, B.J. Nickoloff, F.O. Nestle.

p. ; cm.—(Current directions in autoimmunity, ISSN 1422–2132 ; v.

10)

Includes bibliographical references and index.

ISBN 978–3–8055–8391–6 (hard cover : alk. paper)

1. Skin–Diseases–Immunological aspects. 2. Autoimmune diseases. I.

Nickoloff, Brian J., 1953– II. Nestle, F.O. (Frank O.) III. Series.

[DNLM: 1. Skin Diseases–immunology. 2. Immune System Diseases. W1

CU788DR v.10 2008 / WR 140 D4302 2008]

RL97.D475 2008

616.5'071–dc22

2008000638

Bibliographic Indices. This publication is listed in bibliographic services, including Current Contents® and Index Medicus.

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www.karger.com

Printed in Switzerland on acid-free and non-aging paper (ISO 9706) by Reinhardt Druck, Basel

ISSN 1422–2132

ISBN 978–3–8055–8391–6, eISBN 978–3–8055–8392–3

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Foreword

Autoimmune diseases can affect many different organs, and take years to diagnose because of the myriad symptoms and complex patterns of inflammation gone awry. Human skin, being the largest organ of the body, is frequently not only a primary anatomic site for autoimmune diseases, but also a reflection of autoimmune disease processes based primarily in more deeply seated and less visible extracutaneous tissue locations. Despite the many challenges involved in understanding the complex interplay of genetic and environmental factors that contribute to emergence and perpetuation of dermatological autoimmune diseases, significant progress in delineating the basic science underpinnings of these complicated diseases has been accomplished, leading to more rational therapeutic interventions for many such diseases.

This current volume contains a series of review articles covering autoimmune disease processes of and in skin, featuring disorders of humoral as well as cellular immunity. Indeed, the skin may be viewed as containing a complicated peripheral type of immune system with a confederacy of cells belonging to many lineages, including T cells, natural killer T cells, dendritic cells, macrophages, mast cells, B cells and nerve endings. It should, therefore, not be too surprising that such a bewildering array of disease processes is so frequently detected in the skin. It should also be noted by the inclusion of various diseases within this volume that we are adapting the broadest definition of autoimmunity as possible, namely the presence of chronic inflammation in the absence of a known pathogen. For each chapter, the authors who represent some of the most active researchers working on autoimmune disease processes provide an in-depth analysis of the pathophysiology of their respective diseases,

and fill in as many cellular and molecular mechanistic details that are currently known for each disease process. To complement the specific disease focus, overviews of the skin as an active immune organ and of the immune privilege status of the skin are included. Given the rapid progress and convergence of the fields of molecular genetics and immunology, it is hoped that this volume will contribute to the ongoing intensive efforts to better understand the large number of debilitating diseases that frequently result from autoimmunity, not only in the skin, but beyond to the many other tissue locations where dysregulated inflammatory reactions wreak so much havoc, and negatively impact the quality and duration of human life.

This book is recommended for basic and clinical researchers, as well as students, who wish to better understand the role of the immune system in the pathogenesis and therapeutic approaches for autoimmune diseases from a dermatological perspective. As there are chapters covering the important pathological roles for both T lymphocytes as well as B lymphocytes, the interested reader will find this current volume to represent a comprehensive and up-to-date publication.

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Allergic Contact Dermatitis

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Abstract

Allergic contact dermatitis is a classic example of a cell mediated hypersensitivity reaction in the skin. This occurs as a result of xenobiotic chemicals penetrating into the skin, chemically reacting with self proteins, eventually resulting in a hapten-specific immune response. It is precisely because of this localized immune response that allergic signs and symptoms occur (redness, edema, warmth and pruritus). It has been known for years that conventional T-cells (CD4+ or CD8+ T-cells that express a T-cell receptor α/β) are critical effectors for this reaction. There is emerging evidence that innate immune lymphocytes such as invariant Natural killer T-cells and even Natural killer cells may play important role. Other T-cell types such as Tregulatory cells and the IL-10 secreting Tregulatory cells type 1 are likely to be important in the control (resolution) of allergic contact dermatitis. Other cell types that may contribute include B-cells and hapten-specific IgM. Additionally, epidermal Langerhans cells have been ascribed an indispensable role as an antigen presenting cell to educate T-cells of the skin immune system. Studies of mice that lack this cell type suggest that Langerhans cells may be dispensable, and may even play a regulatory role in allergic contact dermatitis. The identity of the antigen presenting cells that complement Langerhans cells has yet to be identified. Lastly, Keratinocytes play a role in all phases of allergic contact dermatitis, from the early initiation phase with the elaboration of inflammatory cytokines, that plays a role in Langerhans cell migration, and T-cell trafficking, through the height of the inflammatory phase with direct interactions with epidermotrophic T-cells, through the resolution phase of allergic contact dermatitis with the production of anti-inflammatory cytokines and tolerogenic antigen presentation to effector T-cells. As the understanding of allergic contact dermatitis continues to improve, this will provide novel therapeutic targets for immune modulating therapy.

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Allergic contact dermatitis (ACD) is a common inflammatory skin disease. Its etiology can be suggested by the body sites of involvement, and potentially confirmed by diagnostic patch testing [1]. This clinically useful test to diagnose ACD reiterates the elicitation phase of ACD, while the afferent phase

that occurs in patients with this condition develops gradually over time as a result of repeated, low-grade exposures to the offending chemicals. While there are many possible conditions that can cause clinical findings of dermatitis, ACD is a distinct entity, and its mechanisms of initiation, amplification, plateau phase and resolution are well studied. Despite all that is known about ACD, there remains a great deal to be learned about this condition. This overview will summarize the findings related to the Immunological mechanisms of ACD. The immunology of ACD is important from the standpoint of the study of a common allergic disease that affects millions of individuals worldwide, and thus is crucial to understand from the public health perspective, and as an important occupational disease. The study of ACD has resulted in advances in the understanding of the skin immune system, which is broadly applicable to other immunological processes that occur in the skin (both protective and disease causing) as well as in other organs/tissues (table 1).

The mechanisms of ACD involve a cascade of complex immune-mediated processes made up of two distinct phases in response to exposure to environmental chemicals, the induction phase (also known as afferent or primary) and the elicitation phase (also known as efferent or secondary; fig. 1).

Although most environmental agents are too large to penetrate into the skin through the stratum corneum, some are of sufficiently low molecular weight to penetrate through this barrier. These molecules can be derived from naturally occurring substances, such as urushiol found in the resin of poison ivy, synthetic compounds and heavy metal ions. Alone, their small size precludes haptens from being efficiently recognized by the immune system. Landsteiner and Jacobs [2] first hypothesized the immune recognition of haptens, based on their studies of 2,4-dinitro-1-chlorobenzene sensitization in guinea pigs. This model involves a process in which haptens chemically react to endogenous proteins within the skin. It has been referred to as immune recognition of 'altered self'. That is, chemical alteration of self-molecules by xenobiotic haptens renders such self-molecules antigenic, in that this neoantigen generated by the hapten-modified self-molecule can elicit a specific immune response. The chemical reaction occurs between the electrophilic components of the hapten and the nucleophilic side chains of the target proteins within the skin. Examples of chemicals containing electrophilic components include aldehydes, ketones, amides or types of polarized bonds. Metal cations (such as Ni^{2+} , one of the most common ACD-associated haptens) are also well-known electrophiles. The most reactive amino acid nucleophilic side chains are those found on lysine, cysteine and histidine. However, their degree of ionization and hence nucleophilicity is dependent on the pH of the microenvironment which is influenced by surrounding amino acids as well as protein location within the epithelium [3]. Predicting the chemicals that can function as haptens in ACD as

Table 1. ACD as a paradigm for protective immunity and pathologic processes in the skin

Physiology/disease state	Comment
Delayed-type hypersensitivity	Significant parallels in that T helper type 1 cells dominate; antigen capture processing and presentation to T cells in the local lymph node are involved. This condition usually involves intradermal exposure to soluble polypeptides; different antigen-presenting cell populations may be involved.
UV immunosuppression	ACD is lost as an early cellular event in this pathologic process; the skin immune system is targeted by the deleterious effects of UV (DNA damage to antigen-presenting cells and other cell types in the skin). ACD is used as a marker for the integrity of the skin immune system.
Percutaneous vaccination	Many parallels between cutaneous delayed-type hypersensitivity and ACD in that cutaneous dendritic cells traffic vaccine components (either a live attenuated pathogen or an antigenic moiety derived from a pathogen) to the skin immune system to elicit protective systemic immunity in the form of cellular and/or humoral immunity.
Antitumor immunity	Cutaneous dendritic cells engulf apoptotic fragments of tumor cells, mature, and traffic these fragments to local lymph node for cross-presentation of tumor antigens to CD8+ and CD4+ T cells for a protective cell-mediated immune response. In disease states such as UV immunosuppression, or tumors with significant resistance mechanisms, this protective system can be subverted.
Antiviral immunity	Cutaneous dendritic cells (Langerhans cells and plasmacytoid dendritic cells) express Toll-like receptors that sense viral infections (for instance, TLR9 that senses herpes simplex virus infection). The dendritic cells become activated, migrate to the local lymph node, secrete type I interferons and inflammatory cytokines such as IL-12, and activate an effective antiviral immune response with predominately CD8+ T cells that secrete IFN- γ .
Autoimmunity-/immune-mediated inflammatory skin diseases	In this setting, cutaneous antigen-presenting cells incite autoimmune attack by either self-reactive immune effector cells (T and/or B cells) or by trafficking self-antigens from antigens from the skin to the skin immune system. This can then initiate the autoimmune cascade and result in a chronic inflammatory process.

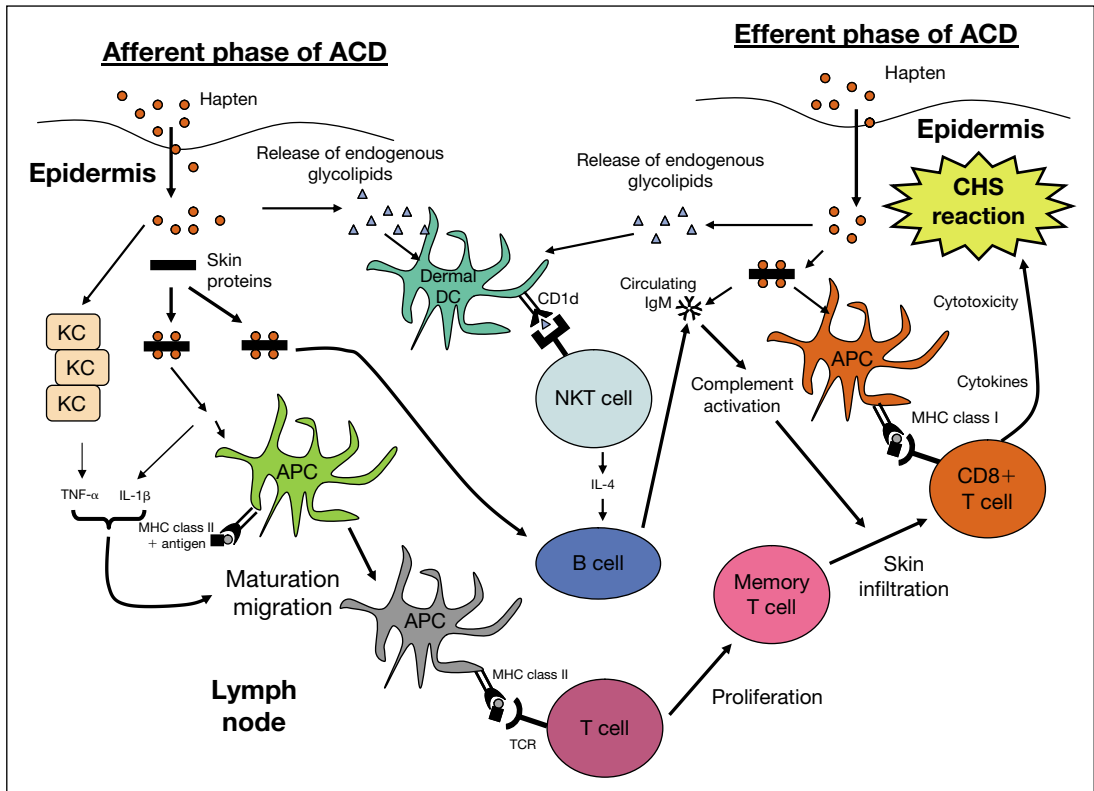


Fig. 1. Schematic representation of the sensitization and elicitation phases of ACD. During the afferent phase of ACD, haptens applied to the skin interact with cellular proteins to form hapten-protein complexes (antigenic moiety recognized by the immune system), which are engulfed by antigen-presenting cells (APC), such as Langerhans cells (LC), and presented in the context of MHC class II. Cutaneous hapten application activates keratinocytes (KC) to release cytokines, such as $\text{TNF-}\alpha$, that function along with APC-derived $\text{IL-1}\beta$ to promote APC maturation and migration to local lymph nodes. In the local lymph nodes the LC activate antigen-specific T cells to proliferate into memory T cells. The cutaneous hapten application also leads to the release of uncharacterized endogenous glycolipids which are presented by CD1d-bearing APC, such as dermal dendritic cells (DC), to natural killer T (NKT) cells leading to the release of IL-4 . B-1 cells in the presence of IL-4 and antigen become activated and release circulating IgM. During the elicitation phase, IgM interacts with the hapten-protein complex to induce complement activation leading to the release of inflammatory and chemotactic factors from mast cells and endothelial cells. Consequently, antigen-specific CD8^+ T cells migrate to the site of hapten application and interact with local APC, resulting in the clinical manifestations of ACD, which is the result of inflammatory cytokines, and cell-mediated cytotoxicity. CHS = Contact hypersensitivity.

well as identifying cutaneous proteins involved in hapten-protein complexes is the subject of intense investigation. One complicating factor is that chemicals that are not normally electrophilic can be converted to a molecule containing properties consistent with haptens by air oxidation or cutaneous metabolism [3]. The role of any given hapten in ACD sensitization is also dependent on its three-dimensional chemical structure, lipophilicity and protein binding affinity. Furthermore, hapten exposure dose, duration and the host magnitude of the immune response are crucial components that result in the development of ACD, which is really a hapten-specific immune response.

Components of the Skin Immune System

Langerhans Cells

A central component of the epithelial immune system are a specific subset of dendritic cells (DC) known as Langerhans cells (LC). The existence of LC was first identified by Paul Langerhans in 1868 [4]. Although originally thought to be neurons based on their staining properties (gold chloride staining, which was used at the time to stain neurons) and cellular morphology, LC subsequently were surmised to function as antigen-presenting cells (APC) [5]. Recent studies of LC indicate that this cell type indeed has direct epidermal innervation and can respond to a number of neurotransmitters that may dramatically affect their immune function [6]. It is generally accepted that the primary function of LC is related to host defense, that is, immunity. LC are the only cell type in normal skin to constitutively express major histocompatibility complex (MHC) class II, a critical restriction element for CD4+ T lymphocytes. This molecule is expressed by all bone marrow-derived APC [7, 8].

LC, in their immature state, are highly efficient at capturing antigens, engulfing them, and processing them for presentation to T lymphocytes after the migration of LC from the epidermis to the local lymph node. This is precisely what is thought to be occurring during ACD. With the initiation of contact dermatitis, the hapten-protein complex (antigen) is engulfed and processed by LC. The LC possess multiple dendritic processes and are organized in a network within the epithelium to ensure efficient trapping of antigens; this has been termed the reticuloepithelial network.

Transgenic mice were created in which endogenous class II MHC (I-A β chain), labeled with enhanced green fluorescent protein, was expressed by epidermal LC as well as other class II MHC-expressing cells. Confocal microscopy monitored in real time the migration of LC over time [9]. At steady state, the majority of LC exhibited relatively little mobility; however, after application of hapten, the majority rhythmically extended and retracted their

dendrites through the intracellular spaces between keratinocytes (KC) in a behavior termed dendrite surveillance extension and retraction cycling habitude (dSEARCH) [10]. Hapten application also induced lateral migration of LC [10]. The amplification of dSEARCH is mediated by interleukin (IL)-1 α and tumor necrosis factor (TNF)- α , cytokines produced by KC [11]. Future studies looking at the uptake of fluorescently labeled particles will start to address correlation of dSEARCH with immunologic function [11].

After cutaneous exposure to the sensitizing hapten, epidermal LC density decreases by approximately 50% at 24 h after exposure [12–14], which occurs due to LC migration from the epidermis to local lymph nodes through dermal lymphatic vessels [14–16]. Lymph nodes of mice sensitized with a contact allergen 24 h before contain LC and can transfer sensitization after implantation into allergen-naïve mice, therefore supporting the role of LC during the initiation phase [17]. LC also undergo maturation to become more efficient APC. In addition to changes in cell morphology and a decreased ability to capture additional antigen, LC exhibit increased expression of CD83 (a marker for LC maturation), adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and costimulatory molecules, including CD40, CD80 and CD86 [17–21]. The expression of these markers is specific to hapten-exposed LC, since dermal irritants, which also trigger LC migration, do not result in similar LC surface marker changes [21]. The increased expression of these signaling molecules on the cell surface of LC is important for efficient activation/proliferation of T cells in the local lymph nodes [22]. After their education by LC, hapten-specific T cells circulate throughout the body, and during the elicitation phase migrate to the epithelium to respond to reexposure of the hapten. Thus, although hapten sensitization is a local event, the memory/effector T cells that develop disseminate, so that this immunologic memory is systemic.

LC migration from the epidermis to local lymph nodes is a highly complex process involving cytokines and chemokines. Intradermal injection of TNF- α triggers LC migration, while TNF- α -neutralizing antibodies blocked hapten-induced LC migration, indicating that LC migration in response to hapten exposure is mediated by TNF- α [23–25]. LC exclusively express the TNF receptor type 2 (TNFR2) [26]. TNFR2 do not contain death domains and interact with c-IAP, a protein that inhibits caspase activity, thereby protecting TNFR2 expressing LC from TNF- α -mediated cell death [26, 27]. This was supported by studies using TNFR knockout mice which indicated LC migration is mediated by TNFR2 but not TNFR1 [28].

IL-1 is part of a family of inflammatory cytokine members that include IL-1 α , IL-1 β and IL-18. All of these cytokines are known to be involved in LC migration in response to hapten exposure during the sensitization phase [29–31]. Of this family of cytokines, IL-1 β is particularly important. It is rapidly produced

by LC after hapten exposure and acts on local KC to produce TNF- α [32]. IL-1 β also acts in an autocrine fashion to provide a required signal for efficient LC migration with TNF- α serving as the second signal [33]. Gene knockout mice in which IL-1 β , TNF- α or TNFR2 genes are not expressed exhibit decreased ACD responses, which supports the conclusion that both TNF- α and IL-1 β signals are required for LC migration during the initiation phase of ACD [34, 35].

In addition to cytokines, chemokines/chemokine receptors modulate LC migration from the dermis to lymphatic vessels and lymph nodes. Immature LC expresses CCR5 and CCR6, two CC chemokine receptors. In response to hapten exposure, LC upregulate CCR7, a CC chemokine receptor that is important for targeting LC to the lymph nodes through binding the CC ligands CCL19 and CCL21 [36, 37]. CCL19 and CCL21 are expressed in the lymph node paracortex and CCL21 is expressed by afferent lymphatic endothelial cells, resulting in a gradient that attracts the LC to the lymph node [38]. CCR7 knockout mice fail to develop contact hypersensitivity (CHS) response, and treatment of normal mice with neutralizing antibodies against CCR7, CCL19 or CCL21 blocks LC migration to the draining lymph node in response to hapten exposure [39, 40].

During the elicitation phase, haptens enter the epithelium and react with endogenous proteins as in the initiation phase. The hapten-protein complexes are then taken up by APC, processed and presented to the antigen-primed T cells in the epithelium and in the dermis. Although LC clearly are capable of functioning as APC, there is evidence indicating they are not required during the elicitation phase of ACD. In the murine model of ACD, trinitrochlorobenzene (TNCB)-sensitized mice were treated with topical steroids for 4 days on their ear, causing a marked reduction (85%) in the epidermal LC density compared to matched controls. When TNCB was then applied to the LC-depleted ears, a paradoxical increased CHS response compared to matched controls was reported as measured by ear swelling [41]. Similar results were obtained when LC were depleted by other methods such as UVB irradiation [41]. The observed increase in TNCB-induced CHS in steroid-treated skin may be related to the increased expression of costimulatory molecules such as CD86. The data suggest that LC are not necessary in the elicitation phase of ACD. Other cell types that may function as APC include mast cells, infiltrating macrophage or KC [42]. Indeed, KC have been shown to express MHC class II and exhibit APC-like properties in response to hapten exposure [43]. The identity of APC responsible for the elicitation phase of ACD and the role LC play are still under investigation.

Epidermal LC: A Role in Immunity or Maintaining Peripheral Tolerance?

Many of the original studies evaluated the role of LC by nonspecific depletion through exposure to UV irradiation or topical glucocorticosteroids, which

are known to deplete epidermal LC. However, these methods also perturb other cutaneous immune cells capable of influencing the immune response, raising the possibility that LC may not be the sole purveyor of ACD.

Three different transgenic mouse models of ACD have questioned the original paradigm of LC as the primary APC in ACD. Bennett et al. [44] used transgenic mice in which the human diphtheria toxin receptor was fused to enhanced green fluorescent protein (to allow for identification of the transgenic cells) and under the control of the langerin gene promoter (knockin mice). Langerin is a C-type lectin specifically expressed on LC [45]. Diphtheria toxin administration in these transgenic mice transiently eliminates LC without harming other cells, since murine cells are resistant to cytotoxicity induced by diphtheria toxin [45–47]. Despite the depletion of LC prior to TNCB exposure, the intensity and kinetics of ACD in mice challenged with TNCB were not significantly different than those of wild-type mice, suggesting that LC were dispensable for ACD [44]. A second study with the same knockin diphtheria toxin receptor mice showed no difference in CHS response to 2,4-dinitrofluorobenzene (DNFB) challenge in LC-depleted animals as compared to wild type [47]. LC depletion after exposure to hapten did not alter the ear swelling response after hapten challenge, again indicating that LC are not necessary for the elicitation phases of ACD [47].

One confounding factor in using the knockin mice is that all langerin-positive cells are affected. As a result, langerin-positive and CD8+ DC found in the draining lymph nodes but not of epidermal LC origin became substantially depleted in this system [48]. Other langerin-positive DC, also unrelated to epidermal LC, such as those found in the spleen and mesenteric lymph nodes, were also depleted by diphtheria toxin administration in this system [48]. Kaplan et al. [49] generated transgenic mice that contain the human langerin gene (huLangerin) and promoter using a bacterial artificial chromosome transgenic system. Human langerin was only expressed in LC and not other DC populations within the draining lymph nodes or spleen [49]. After identifying the selectivity of this system, a second set of transgenic mice was created in which the diphtheria toxin subunit A gene was inserted into the 3' untranslated region of the human langerin gene (huLangerin-DTA). These transgenic mice were selectively LC depleted. DNFB sensitization of such mice resulted in an enhanced CHS response compared to controls (huLangerin transgenic animals, not depleted of LC) [49]. Adoptive transfer experiments addressed whether the increased CHS response in LC-depleted animals occurs during the initiation or elicitation phases. Enhanced CHS as a result of LC selective depletion occurs during the initiation phase and not the elicitation phase. This elegant research alters the classic view of LC in that it demonstrates that LC are not required for antigen presentation but instead appear to have a regulatory function.

KC: A Key Participant in ACD

KC are a critical cell type of the epidermis because of their numeral dominance and their role in the formation of the anatomic barrier function of the skin. As well, they play a critical role in skin immunity and the pathogenesis of ACD. KC are the source of the cutaneous TNF- α after hapten exposure, and thus play a critical role in modulating LC maturation and migration from the epidermis to the local lymph node [50]. As described earlier, they are important in their interactions, via cytokines, in the two-signal model of LC migration. KC IL-1 receptors respond to LC-derived IL-1 β , which induces TNF- α , the necessary second signal for LC activation and migration [33]. IFN- γ induces KC to express ICAM-1 [51]. IFN- γ producing T cells express CD11a which binds to ICAM-1 (CD54) on KC, thereby facilitating T cell infiltration into the epidermis [52]. This suggests that KC play an active role in the pathogenesis of ACD by aiding lymphocyte infiltration.

IFN- γ also induces the expression of class II MHC by KC, providing this cell type with the recognition molecules to present to CD4⁺ T lymphocytes [53]. However, unlike professional APC, KC only minimally express CD80 or CD86. These molecules are important second signals that allow activation of the antigen-specific immune response via its receptors on T cells [CD28/cytotoxic T lymphocyte-associated antigen-4 (CTLA-4)]. In the absence of CD80/CD86, antigen presentation in the context of MHC class II leads to clonal anergy, a type of immunological unresponsiveness, in which T cells are viable but unable to receive antigen-specific signals through their T cell receptor.

KC may function to limit the amplitude and duration of ACD response by inducing CD4⁺ T cell clonal anergy (fig. 2). They may compete with professional, bone marrow-derived APC to present hapten to T cells that migrate into the skin, particularly within the epidermis. Thus, if a hapten-specific CD4⁺ T lymphocyte encounters a class II MHC bearing APC such as an LC, it will be activated, proliferate and clonally expand. In contrast, a class II MHC bearing KC will induce a state of clonal anergy. Such tolerized T cells express high levels of IL-2 receptors, and can thus compete with fully activated T cells for this critical growth factor. This may serve to limit ACD. To address the hypothesis related to tolerance induction, transgenic mice were created that constitutively express high levels of CD80 or CD86 specifically in KC using the keratin 14 promoter. Cutaneous hapten application resulted in a prolonged CHS response in previously sensitized mice lasting up to 8 weeks in the transgenic animals, while the wild-type mice exhibited a normal CHS response lasting 24 h [54, 55]. These data are consistent with the hypothesis that KC dampen CHS and play a role in its resolution. Recent studies found that the CD80 promoter is activated in the presence of the prototypical contact allergen nickel chloride at levels similar to professional APC [56]. These data suggest that individuals who do not develop ACD to hapten

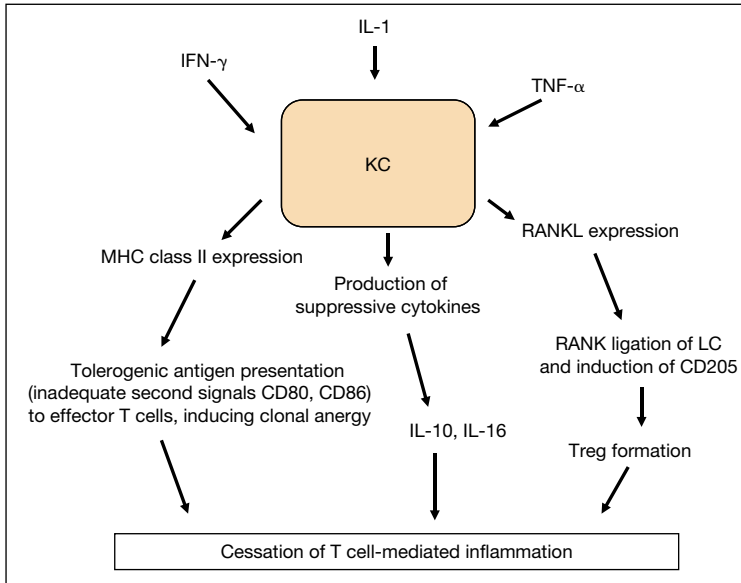


Fig. 2. Keratinocyte (KC) tolerance cascades dampen T cell-mediated inflammation (such as ACD) in the skin. In the presence of inflammatory cytokines such as IFN- γ , TNF- α and IL-1, KC upregulate MHC class II molecules. However, they do not upregulate the costimulatory molecules such as CD80 or CD86, which are required for T cell activation, resulting in T cell anergy. Transgenic mice with KC that constitutively express CD86 have significantly prolonged contact hypersensitivity (CHS) reactions [54]. KC also upregulate receptor activator of nuclear factor- κ B (RANK) ligand (RANKL) which interacts with RANK on Langerhans cells (LC). This interaction triggers upregulation of CD205 and ultimately leads to activation of T regulatory cells (Treg). Tregs function to suppress the inflammatory response through the release of cytokines such as IL-10 and TGF- β , leading to the resolution of ACD and may be the mechanism of hapten tolerance. KC also secrete cytokines important in suppressing ACD, such as IL-10 and IL-16.

exposure may possess KC that express only negligible levels of CD80/CD86, suggesting that the amplitude of KC costimulation may be relevant for the susceptibility to allergic sensitivity processes. Ongoing studies are designed to address this hypothesis.

Other findings support the conclusion that KC are important for suppressing CHS responses via IL-10 secretion, particularly in response to hapten exposure [57, 58]. This cytokine suppresses CHS responses. KC have also been shown to produce IL-16 in response to hapten exposure [59], which is a cytokine that is implicated in immune cell chemotaxis, suggesting it may be involved

during the elicitation phase of ACD. Interestingly, IL-16 only first appears 6 h after hapten exposure, with maximal expression at 24 h after exposure during the elicitation phase. The primary infiltrating T cells during the elicitation phase, CD8+ T cells, appear in the skin earlier than 6 h after hapten exposure (see T Lymphocytes: Critical Effectors and Regulators of CHS below). IL-16 has been specifically associated with CD4+ T cell chemotaxis [60] and the expression of IL-16 coincides with CD4+ T cell infiltration [59]. These CD4+ T cells are not inflammatory, rather they function to downregulate the CHS response, supporting the finding that KC are involved in suppression of ACD.

KC express high levels of receptor activator of nuclear factor κ B ligand (RANKL) in response to inflammation [61]. Its receptor RANK is found on LC, leading to upregulation of cell surface markers, including CD205 and CD86 [61]. CD205 expression on DC is associated with induction of CD4+ CD25+ T cells that suppress the immune response [62]. Indeed, upregulation of RANKL by KC resulted in increased levels of CD4+ CD25+ T cells through an LC-dependent mechanism [61]. UV light is known to suppress CHS responses, and is also a potent inducer of RANKL on KC [61, 63]. All these observations support the concept that KC are central regulators of T cell-mediated immunity in ACD, as well as other immunologic reactions in the skin. KC facilitate immune cell infiltration important for the elicitation phase of ACD, but more importantly appear to promote the resolution of the inflammatory response by secreting IL-10 and initiating T regulatory cell (Treg) activation. Thus, chronic inflammatory skin diseases (perhaps chronic dermatitis) may involve pathological alterations in KC ability to induce tolerance and/or promote the resolution of T cell-mediated inflammation in the skin.

Lymphocyte Populations Involved in ACD

T Lymphocytes: Critical Effectors and Regulators of CHS

Hapten-specific T cells in the local lymph nodes are primed by hapten-bearing APC, and undergo clonal expansion during the sensitization phase. These T cells then circulate throughout the blood and are recruited to the skin upon reexposure of the same hapten. The T cell-mediated inflammatory reaction typical of the elicitation phase of ACD occurs 48–72 h after exposure to hapten, giving the appearance of a delayed-type hypersensitivity (DTH) reaction. However, unlike classic DTH where the effector cells are CD4+ T cells, the primary effector cells of ACD are CD8+ T cells [64–67]. This was first demonstrated using in vivo depletion of CD4+ or CD8+ T cells with monoclonal antibodies in a mouse model of ACD [74]. A second study using gene-targeted mice confirmed these results. MHC class I knockout mice, which are deficient

in CD8+ T cells, did not develop a CHS reaction to DNFB cutaneous application. Conversely, MHC class II-deficient mice, which are deficient in CD4+ T cells, developed a strong CHS reaction to DNFB, supporting the conclusion that CD8+ T cells are primed in the absence of CD4+ T cells and mediate the CHS response [67]. Using CD4 gene-targeted mice, as opposed to MHC class II-deficient mice, gives contradictory results. The CD4 gene-targeted mice exhibited a significantly decreased CHS response to DNFB [68]. Later studies found that the impaired CHS response in these mice is in fact due to impaired hapten-specific CD8+ T cell function [69]. Conversely, MHC class II-deficient mice are deficient in CD4+ T cells without altering the function of the CD8+ T cells [70].

The majority of studies supports the conclusion that CD8+ T cells are the effector cells during elicitation of ACD [70]. Interestingly, trinitrophenyl (TNP), a strong hapten that also induced a predominantly CD8+ T cell CHS response in normal mice, triggered a normal CHS response in CD8+ T cell-depleted mice. This was blocked by depletion of CD4+ T cells, indicating that in the absence of CD8+ T cells, CD4+ T cells are capable of mediating the CHS to TNP [70]. TNP has been shown to activate both CD4+ and CD8+ T cells and in vitro studies indicated that hapten-specific CD8+ T cells induce Fas-mediated apoptosis of CD4+ T cells [70]. Therefore, it is possible that during sensitization, CD8+ T cells trigger apoptosis of CD4+ T cells, thereby eliminating hapten-specific CD4+ T cell priming/expansion and ensuring that the CD8+ T cells are the dominant effector cells [71].

Although CD4+ T helper (Th) cells are not central in the pathogenesis of ACD, cytokines involved in Th1 cell proliferation/activation are important in the development of CHS responses. Much of this is based on finding that IL-12 exacerbates CHS reactions by suppressing the production of regulatory cytokines such as IL-4 and IL-10 [72]. APC produce IL-12 (including LC) and promote T cell development to favor Th1 cells by upregulating IFN- γ in T cells [73]. Furthermore, contact allergens triggered KC to produce IL-12 leading to proliferation of T cells into a Th1 phenotype [74]. In vivo studies demonstrated that both initiation and elicitation phases of DNFB-mediated ACD were significantly blocked by IL-12-neutralizing antibodies, supporting the conclusion that IL-12 is important in the pathogenesis of ACD [75]. Interestingly, IL-12 gene-targeted mice exhibited no change in DNFB-mediated ACD [76]. One possible explanation is that cytokines have the potential for redundant function so that the complete deletion of IL-12 during development is compensated by other cytokines [38].

IFN- γ is classically produced by Th1 CD4+ cells, but in CHS has been demonstrated to be produced by the CD8+ T cells [77]. This cytokine is an important component of the inflammatory response, because it induces

mononuclear cell infiltration, triggers upregulation of ICAM-1, a molecule important in chemotaxis, and induces increased expression of MHC class I, a molecule important in antigen presentation [78]. Gene-targeted mice that do not express the IFN- γ receptor do not exhibit alteration in cutaneous edema typical of CHS by a strong sensitizer such as TNCB. However, these mice did exhibit significant downregulation of inflammatory infiltrate, supporting the conclusion that IFN- γ is important in the pathogenesis of cellular infiltration associated with CHS, while cutaneous edema is IFN- γ independent [78], suggesting that the function of IFN- γ may overlap with other cytokines [78]. Numerous other cytokines have been recently identified as potentially involved in the pathogenesis of ACD. For a complete list of cytokines associated with the pathogenesis of ACD, see table 2.

B Lymphocytes: An Unappreciated Participant in ACD

Much of the early studies examining the mechanism of ACD focused primarily on the role of CD4+ versus CD8+ T cells and LC. As a cell-mediated immune hypersensitivity reaction process similar to DTH, ACD was assumed to be a B cell-/antibody-independent process in which the presence of hapten-protein complexes presented in the context of MHC class II by APC is sufficient to trigger the production of cytokines and recruit activated T cells leading to the inflammatory response of ACD. Recent studies provided evidence of the contrary. Mice deficient in C5, a component of the complement system, exhibited significantly impaired CHS in response to topical application of the hapten picryl chloride (PCI) [79]. Similar results were found after administration of either sCR1, a complement-blocking reagent, or anti-C5 antibody, while PCI-induced CHS was restored after injection of normal mouse serum (containing high titers of C5), supporting the conclusion that C5 is required for CHS [79, 80]. Furthermore, gene-targeted mice, in which the receptor for activated C5 (C5a) was genetically deleted, resulted in decreased CHS responses and leukocyte chemotaxis [81]. The facts that (1) C5a is well known to mediate leukocyte chemotaxis and stimulate local inflammatory responses, and (2) antibodies are important in triggering complement activation, indicate that B cells are important contributors in the pathogenesis of CHS [82, 83].

B-1 and B-2 subsets of antibody-producing B lymphocytes have been recognized. B-1 cells are T cell independent, do not form germinal centers, generally do not undergo isotype DNA rearrangements and are the source of IgM antibodies found in normal serum [84]. B-2 cells are the classic B cells that recirculate to peripheral lymphoid organs, undergo T cell-dependent isotype rearrangements, and produce all subclasses of antibodies. B-1 cell-deficient mice exhibited significantly decreased CHS responses to PCI, which was restored with antigen-specific monoclonal IgM, adoptive transfer of B-1 cells

Table 2. Critical cytokines and chemokines of ACD

Cytokine/chemokine	Function in ACD
IL-1 α	Proinflammatory cytokine secreted by KC; important in LC migration and activation of hapten-specific T cells; absence in gene-targeted mice diminishes CHS response
IL-1 β	Produced by LC; important for LC migration and maturation by directly acting on LC inducing local KC to produce TNF- α ; absence in gene-targeted mice diminishes CHS response
IL-2	T cell growth factor; produced by CD8+ T cells leading to proliferation of Tregs which in turn feedback to inhibit further activation of CD8+ T cells
IL-3	Role in ACD unknown; involved in normal mast cell development; absence in gene-targeted mice diminishes CHS response
IL-4	Produced by invariant natural killer T cells, necessary for B-1 cell activation and the production of IgM antibody; absence in gene-targeted mice diminishes CHS response
IL-5	Required for maintenance of B-1 cells and the production of IgM antibody; absence in gene-targeted mice diminishes CHS response
IL-6	Primary inflammatory cytokine produced by DC and involved in T cell proliferation in local lymph nodes following primary sensitization; absence in gene-targeted mice diminishes CHS response
IL-8	T cell chemotaxis; induced by TNF- α
IL-10	Produced by Treg and KC; functions to suppress inflammatory response; absence in gene-targeted mice enhances CHS response
IL-12	Critical cytokine in ACD; produced by LC, leads to IFN- γ production by Th1 cells; also protects against UV immunosuppression.
IL-16	Produced by KC after hapten exposure; involved in recruitment of CD4+ T cells (likely Th2 or Tregs); neutralizing monoclonal antibody against IL-16 enhances CHS response
IL-17	Produced by CD8+ effector T cells; important during elicitation; absence in gene-targeted mice diminishes CHS response

IL-18	Involved in the initiation phase of ACD; biologic properties similar to IL-12; produced by LC and leads to production of IFN- γ ; absence in gene-targeted mice diminishes CHS response
IL-31	Th2 cytokine significantly increased in ACD; unknown function
TNF- α	Primary inflammatory cytokine; important in LC migration and maturation, produced by KC after hapten exposure; absence in gene-targeted mice diminishes CHS response
IFN- γ	Produced by CD8+ T cells (Tc1) and CD4+ effector T cells (Th1); important in the pathogenesis of cellular infiltration into skin.
TGF- β	Produced by a subset of T cells and involved in tolerance; downregulates IL-1 receptor expression; administration of recombinant TGF- β during elicitation phase blocks CHS response
MIP-2	Inflammatory cell chemotaxis into skin
GM-CSF	Important in LC maturation; involved in upregulation of MHC class II and B7 molecules
MCP-1	LC migration and amplifies T cell proliferation
RANTES	Chemotaxis of T cell into skin; induced by TNF- α
MIF	Involved in LC migration and T cell proliferative responses

MIP = Macrophage inflammatory protein; GM-CSF = granulocyte-macrophage colony-stimulating factor; MCP = monocyte chemoattractant protein; RANTES = regulation on activation, normal T cell expressed and secreted; MIF = macrophage migration inhibitory factor.

or administration of serum from wild-type mice 24 h after sensitization [85]. During the afferent phase of CHS, B-1 cells rapidly (within 24 h) proliferate and produce IgM, while B-2 cells remain at preexposure levels, supporting the conclusion that IgM antibody produced by B-1 cells is required for CHS responses [85]. It is important to note that sensitized circulating T lymphocytes are unable to mediate CHS or DTH responses after antigen exposure in the absence of antigen-specific antibodies [86]. Together, the data indicate that in response to hapten, B-1 cells rapidly proliferate and produce circulating IgM antibodies that activate complements. C5a triggers inflammation through binding to C5a receptors on mast cells and platelets (which induces release of inflammatory cytokines such as TNF- α), leading to recruitment of effector T cells [86]. Although B-1 cells are described as T cell independent, a novel subset of T cells known as natural killer T (NKT) cells have been shown to be involved in B-1 cell activation.

NKT Cells: Critical in the Early Cellular and Molecular Events of CHS

NKT cells are a unique lymphocyte subset characterized by their expression of CD161 [also known as NK1.1; classically expressed on natural killer (NK) cells] and the α/β chains of the T cell receptor (TCR). They are considered to be a cellular component of the innate immune response. Although they express a TCR, a large majority of NKT cells do not undergo rearrangement in response to antigen; rather they express an invariant α -chain (V α 14J α 18 in mice and V α 24J α Q in humans) paired with a β -chain consisting of either V β 8 or V β 2 [87]. Because of this invariant TCR expression, their repertoire of antigens is thought to be highly limited. Their TCR bind highly conserved glycolipids in the context of CD1d, an MHC class I-like molecule found on APC. The identity of the glycolipids presented to invariant NKT (iNKT) cells in vivo is still unknown, although isoglobohexoside has been identified as an endogenous ligand recognized by iNKT cells. The glycolipid α -galactosylceramide, derived from a sea sponge, is a strong ligand for activating both human and mouse iNKT and is widely used to study these cells. To date, α -galactosylceramide has not been found in endogenous tissue or on infectious agents, suggesting that other ligands function to activate iNKT cells in vivo [87, 88]. Once activated, iNKT cells secrete cytokines including IL-2, TNF- α , IL-4 or IFN- γ , depending on the local microenvironment. Some iNKT cells also possess cytotoxic activity similar to CD8+ (cytotoxic) T lymphocytes; however, the control of their cytotoxic activity and the role of this activity in vivo is still unclear [89]. NKT cells have been implicated as part of the host defense against infectious agents as well as in the pathogenesis of a wide variety of diseases, including allergic asthma, autoimmune hepatitis, diabetes and lupus [87, 88, 90–93].

NKT cells play an important role in the pathogenesis of ACD. These cells are required for CHS induced by topical PCI using CD1d (required for NKT activation) as well as $\alpha 18$ knockout mice [94]. Cutaneous application of hapten rapidly induced (within 1 h) iNKT cell proliferation in the liver but not in the lymph nodes, spleen or peritoneal cavity. Indeed, adoptive transfer mononuclear cells containing iNKT isolated from the wild-type liver restored the CHS response [94]. Adoptive transfer of wild-type effector T cells (depleted of all B cells) 4 days after hapten exposure failed to induce CHS in CD1d or $\alpha 18$ knockout mice 1 day after hapten exposure. Similar transfer experiments into wild-type animals elicited a strong CHS response. Since B-1 cells, but not antigen-specific effector T cells, are activated 24 h after hapten exposure [85, 95], the inability to elicit a normal CHS response in iNKT cell-deficient mice 24 h after exposure to hapten suggests that iNKT cells are involved in activating B-1 cells. Intravenous administration of IL-4, a cytokine rapidly produced by iNKT cells and known to activate B-1 cells, restored the CHS response in iNKT cell-deficient animals, suggesting the iNKT cell activation of B-1 cells is mediated by secretion of IL-4 [94].

The importance of IL-4 was confirmed in follow-up studies using IL-4 knockout mice in which CHS was impaired but restored with the administration of IL-4 or with adoptive transfer of wild-type B-1 cells harvested 1 day after hapten exposure [96]. Impaired CHS from B cell-deficient mice was only restored with adoptive transfer of B-1 cells from wild-type animals. Adoptive transfer of B-1 cells from mice with IL-4 receptor or STAT6, an intracellular molecule important in IL-4 receptor signaling, gene knockouts was unable to restore the CHS response, indicating that IL-4 signaling is important in B-1 cell activity [96]. To identify whether iNKT cells are the source of IL-4, in vitro experiments were conducted using liver iNKT cells harvested 1 h after cutaneous hapten application. These iNKT cells activated B-1 cells harvested from either wild-type or IL-4 knockout mice exposed to an unrelated hapten, supporting the conclusion that IL-4 is produced by iNKT and not B-1 cells [97]. The production of IL-4 by iNKT cells was shown to be Toll-like receptor dependent [98]. Collectively, the data indicate the iNKT cells are rapidly activated after hapten exposure to produce IL-4, which in turn activates B-1 cells in the presence of hapten to produce IgM. The mechanism involved in selective iNKT cell proliferation/activation in the liver but not other organs in response to cutaneous hapten exposure is still unclear and requires further investigation.

Tregs: A Role in Downregulating ACD

Studies investigating the termination of ACD deserve discussion. Early observations found that intradermal injection of IL-10 suppressed the CHS response to DNFB [99]. IL-10 gene-targeted mice exhibited enhanced CHS; alternatively, wild-type mice treated with neutralizing antibody to IL-10 also

exhibited increased CHS. These data are consistent with the suppressive role of IL-10 in ACD [100, 101]. IL-10, IL-4 and IL-5 are classically considered Th2 cytokines, suggesting that CD4+ T cells are involved in their production. MHC class II knockout mice, which are specifically deficient in CD4+ T cells, developed an enhanced CHS response to the hapten DNFB as compared to wild-type animals, indicating that CD4+ T cells function to downregulate the CHS response [64]. Further studies demonstrated that CD4+ T cell-deficient mice exhibit significantly decreased levels of antigen-specific CD8+ T cells in skin draining lymph nodes after hapten sensitization, which means that CD4+ T cells regulate the development of CD8+ T cells [102]. Interestingly, although Th2 cells are known to produce both IL-4 and IL-10, an early study found that IL-4 was not involved in suppression of ACD, suggesting that cell types other than classic Th2 cells were responsible for production of IL-10 [101].

In recent years, novel CD4+ T cell populations have been described and implicated in the regulation of ACD. The most widely studied of these subsets are the Tregs. In addition to expressing CD4 molecules, Tregs also constitutively express the IL-2 receptor α -chain (CD25) at high levels, CTLA-4 and forkhead box P3 (Foxp3), and the expression of these molecules on CD4+ T cells facilitates the identification of these cells [103–105]. Tregs were originally shown to be important in controlling autoimmunity by suppressing the immune system [103]. Direct cell-to-cell interaction was believed to be the mechanism of their immune suppression function in a variety of diseases, including ACD via molecules such as CTLA-4, which has a high affinity for the costimulatory molecules CD80/CD86 and inhibits IL-2 secretion important for effector T cell proliferation [106]. They can express cutaneous lymphocyte-associated antigen presumably after interaction with DC in the cutaneous draining lymph nodes and migrate to the skin [107]. Tregs are specifically recruited to the skin after hapten exposure. In individuals who do not develop ACD to nickel, the Tregs were able to inhibit effector T cell activation, while individuals who exhibit ACD to nickel were unable to suppress nickel-specific effector T cell activation in vitro, supporting the conclusion that Tregs are involved in ACD suppression and hapten tolerance [108].

A recent study contradicted previously held beliefs by clearly demonstrating that Treg immune suppression of effector T cell activation is not mediated by direct cell-to-cell contact but rather through secretion of suppressive cytokines, specifically IL-10 [109]. Interestingly, a new subtype of Tregs that specifically produce large amounts of IL-10 has been recently identified and isolated from ACD-affected skin [110]. These cells, termed Treg type 1 (Tr1) cells, are distinct from classic Tregs in that they are inducible and secrete copious amounts of IL-10 [111]. Tr1 cells have been shown to block effector T cell activation after hapten exposure through the secretion of IL-10 and are believed to temper the T cell response to cutaneous hapten [112]. Although it is possible

that Treg and Tr1 cells function independently, the cytokine-mediated suppression by Tregs might be mediated through direct activation of Tr1 cells. Indeed, Tregs have been shown to induce IL-10 production in Tr1-like cells, supporting the conclusion that these emerging CD4+ T cell subtypes function together to regulate and suppress the CHS response to hapten [113].

NK Cells: An Emerging Role in CHS

NK cells are a major component of the innate immune system. They do not express a cell surface TCR and classically do not function in an antigen-specific manner, nor are they thought to mediate immunological memory. However, a recent study by O'Leary et al. [114] suggested that NK cells have the ability to acquire hapten-specific memory and mediate CHS. They used recombination activating gene 2 (Rag2) knockout mice which are devoid of T and B cells (Rag2 is required for antigen-specific receptor rearrangement on B and T cells). When these mice were sensitized to DNFB they mounted a vigorous CHS response when exposed to DNFB but not other haptens, indicating that CHS is inducible and hapten specific in the absence of T and B cells. NK cell infiltration to DNFB-exposed skin was rapid and the CHS response was blocked by NK cell depletion. Furthermore, adoptive transfer of NK cells isolated from the livers of DNFB-sensitized Rag2 knockout mice into Rag2 and IL-2 receptor- γ gene-targeted mice (devoid of B, T and NK cells), which are unable to mount a CHS response, resulted in restoration of the CHS response to DNFB. Collectively, the data indicate that in the absence of B and T cells, hepatic NK cells possess hapten-specific memory and can mediate a hapten-specific CHS response [114]. This finding is remarkable because it suggests NK cells, which lack a TCR, have some capacity to develop memory and antigen specificity, a characteristic associated with TCR-bearing conventional T cells. A summary of the cells involved in ACD is shown in table 3.

Conclusions and Future Directions

ACD is a highly complex process that involves the collaboration of the skin immune system locally, with the potential for the dissemination of this local immunity to that of a systemic immune response. The understanding of the underlying cellular and molecular pathogenesis of ACD has expanded dramatically. In addition to classic T cells, relatively new cell types such as NKT and Tregs have emerged as critical participants, while the role of other cell types such as LC, appears to be different than previously believed. LC were thought to be the primary APC required for the initiation and possibly the elicitation phases of ACD, while recent work suggests they are dispensable for CHS

Table 3. Cell types involved in the mechanisms of ACD

Cell type	Function in ACD
LC	Originally thought to be the primary APC; however, new data suggest a regulatory function
KC	Facilitates T cell infiltration into the epidermis by expressing specific receptors that bind to molecules located on T cell surface; involved in the initiation phase of ACD by producing cytokines that mobilize LC to migrate; also involved in termination of ACD through tolerogenic antigen presentation and the production of IL-10 and IL-16, which recruit Tregs
CD8+ T cell	Major effector cell in CHS; source of IFN- γ production
CD4+ T cell	Some experimental data support a role for Th1 memory/effector cells in ACD
B-1 cell	Produces IgM antibody in response to IL-4 leading to complement activation and leukocyte chemotaxis
iNKT cell	Responds to still unidentified endogenous glycolipids after hapten exposure which leads to IL-4 production to activate B-1 cells
Treg11	T cell subset (CD4+, CD25 ^{high} , Foxp3 transcription factor, CTLA-4+) that functions to suppress the T cell-dependent inflammatory response seen in ACD and critical to hapten tolerance; defined by its phenotype as well as its suppressive functions
NK cell	Member of the innate immune system recently shown to exhibit memory and antigen specificity in a model of murine ACD
Mast cell	Produces TNF- α , which induces DC migration; promotes T cell infiltration through release of IL-3; induces their proliferation and activation; releases mediators that promote inflammation; may function in antigen presentation

responses and may play a role in the development of immune tolerance. The role of B cells has also been clarified, as they appear to be important during the initiation of ACD by secreting IgM antibody in response to NKT cell-derived IL-4 leading to complement activation and ultimately immune cell chemotaxis.

The identity of APC during the initiation and elicitation phases is still not known and the cellular mechanism during the early portion of the elicitation phase is not entirely clear. Also, the role of KC is not completely defined. While they appear to be tolerogenic, and play a role in the dampening of CHS, studies of the expression and modulation of costimulatory molecules such as CD40 and CD80 suggest that they may have the capacity to act as facultative amplifying APC for epidermotrophic T lymphocytes. Furthermore, the mechanism of the systemic immune cell activation after cutaneous hapten exposure as well as the exact molecular events leading to antigen tolerance require further investigation. The provocative work that suggests NK cells play a role in ACD needs further confirmation, and has the potential to redefine the role of NK cells in the immune response. As the molecular and cellular pathogenesis of ACD is elucidated, new therapeutic targets for ACD will become available.

References

- 1 Mowad CM: Patch testing: pitfalls and performance. *Curr Opin Allergy Clin Immunol* 2006;6: 340–344.
- 2 Landsteiner K, Jacobs J: Studies on the sensitization of animals with simple chemical compounds. *J Exp Med* 1935;61:643–656.
- 3 Divkovic M, Pease CK, Gerberick GF, et al: Hapten-protein binding: from theory to practical application in the in vitro prediction of skin sensitization. *Contact Dermatitis* 2005;53: 189–200.
- 4 Langerhans P: Über die Nerven der menschlichen Haut. *Virchows Arch Pathol Anat* 1868;44: 325–337.
- 5 Silberberg I: Apposition of mononuclear cells to Langerhans cells in contact allergic reactions: an ultrastructural study. *Acta Derm Venereol* 1973;53:1–12.
- 6 Seiffert K, Granstein RD: Neuroendocrine regulation of skin dendritic cells. *Ann NY Acad Sci* 2006;1088:195–206.
- 7 Jones PP, Murphy DB, Hewgill D, et al: Detection of a common polypeptide chain in I-A and I-E sub-region immunoprecipitates. *Mol Immunol* 1979;16:51–60.
- 8 Matis LA, Glimcher LH, Paul WE, et al: Magnitude of response of histocompatibility-restricted T-cell clones is a function of the product of the concentrations of antigen and Ia molecules. *Proc Natl Acad Sci USA* 1983;80:6019–6023.
- 9 Vishwanath M, Nishibu A, Saeland S, et al: Development of intravital intermittent confocal imaging system for studying Langerhans cell turnover. *J Invest Dermatol* 2006;126:2452–2457.
- 10 Nishibu A, Ward BR, Jester JV, et al: Behavioral responses of epidermal Langerhans cells in situ to local pathological stimuli. *J Invest Dermatol* 2006;126:787–796.
- 11 Nishibu A, Ward BR, Boes M, et al: Roles for IL-1 and TNF α in dynamic behavioral responses of Langerhans cells to topical hapten application. *J Dermatol Sci* 2007;45:23–30.
- 12 Bergstresser PR, Toews GB, Streilein JW: Natural and perturbed distributions of Langerhans cells: responses to ultraviolet light, heterotopic skin grafting, and dinitrofluorobenzene sensitization. *J Invest Dermatol* 1980;75:73–77.

- 13 Weinlich G, Heine M, Stossel H, et al: Entry into afferent lymphatics and maturation in situ of migrating murine cutaneous dendritic cells. *J Invest Dermatol* 1998;110:441–448.
- 14 Steinbrink K, Kolde G, Sorg C, et al: Induction of low zone tolerance to contact allergens in mice does not require functional Langerhans cells. *J Invest Dermatol* 1996;107:243–247.
- 15 Larsen C, Steinman R, Witmer-Pack M, et al: Migration and maturation of Langerhans cells in skin transplants and explants. *J Exp Med* 1990;172:1483–1493.
- 16 Lukas M, Stossel H, Hefel L, et al: Human cutaneous dendritic cells migrate through dermal lymphatic vessels in a skin organ culture model. *J Invest Dermatol* 1996;106:1293–1299.
- 17 Moodycliffe AM, Shreedhar V, Ullrich SE, et al: CD40-CD40 ligand interactions in vivo regulate migration of antigen-bearing dendritic cells from the skin to draining lymph nodes. *J Exp Med* 1999;191:2011–2020.
- 18 Nuriya S, Yagita H, Okumura K, et al: The differential role of CD86 and CD80 co-stimulatory molecules in the induction and the effector phases of contact hypersensitivity. *Int Immunol* 1996;8:917–926.
- 19 Zhou L, Tedder T: A distinct pattern of cytokine gene expression by human CD83+ blood dendritic cells. *Blood* 1995;86:3295–3301.
- 20 Lehe CL, Jacobs JLL, Hua CM, et al: Subtoxic concentrations of allergenic haptens induce LC migration and maturation in a human organotypic skin explant culture model: a novel method for identifying potential contact allergens. *Exp Dermatol* 2006;15:421–431.
- 21 Aiba S, Terunuma A, Manome H, et al: Dendritic cells differently respond to haptens and irritants by their production of cytokines and expression of co-stimulatory molecules. *Eur J Immunol* 1997;27:3031–3038.
- 22 Hauser C: Cultured epidermal Langerhans cells activate effector T cells for contact sensitivity. *J Invest Dermatol* 1990;95:436–440.
- 23 Cumberbatch M, Fielding I, Kimber I: Modulation of epidermal Langerhans' cell frequency by tumour necrosis factor- α . *Immunology* 1994;81:395–401.
- 24 Cumberbatch M, Kimber I: Dermal tumour necrosis factor- α induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhans' cell migration. *Immunology* 1992;75:257–263.
- 25 Cumberbatch M, Kimber I: Tumour necrosis factor- α is required for accumulation of dendritic cells in draining lymph nodes and for optimal contact sensitization. *Immunology* 1995;84:31–35.
- 26 Kimber I, Dearman RJ, Cumberbatch M, et al: Langerhans cells and chemical allergy. *Curr Opin Immunol* 1998;10:614–619.
- 27 Shu HB, Takeuchi M, Goeddel DV: The tumor necrosis factor receptor 2 signal transducers TRAF2 and c-IAP1 are components of the tumor necrosis factor receptor 1 signaling complex. *Proc Natl Acad Sci USA* 1996;93:13973–13978.
- 28 Wang B, Fujisawa H, Zhuang L, et al: Depressed Langerhans cell migration and reduced contact hypersensitivity response in mice lacking TNF receptor p75. *J Immunol* 1997;159:6148–6155.
- 29 Nakae S, Naruse-Nakajima C, Sudo K, et al: IL-1 α , but not IL-1 β , is required for contact-allergen-specific T cell activation during the sensitization phase in contact hypersensitivity. *Int Immunol* 2001;13:1471–1478.
- 30 Cumberbatch M, Dearman RJ, Antonopoulos C, et al: Interleukin (IL)-18 induces Langerhans cell migration by a tumour necrosis factor- α - and IL-1 β - dependent mechanism. *Immunology* 2001;102:323–330.
- 31 Cumberbatch M, Dearman RJ, Kimber I: Interleukin 1 β and the stimulation of Langerhans cell migration: comparisons with tumour necrosis factor α . *Arch Dermatol Res* 1997;289:277–284.
- 32 Enk AH, Angeloni VL, Udey MC, et al: An essential role for Langerhans cell-derived IL-1 β in the initiation of primary immune responses in skin. *J Immunol* 1993;150:3698–3704.
- 33 Stoitzner P, Zanella M, Ortner U, et al: Migration of Langerhans cells and dermal dendritic cells in skin organ cultures: augmentation by TNF- α and IL-1 β . *J Leukoc Biol* 1999;66:462–470.
- 34 Shornick LP, De Togni P, Mariathasan S, et al: Mice deficient in IL-1 β manifest impaired contact hypersensitivity to trinitrochlorobenzene. *J Exp Med* 1996;183:1427–1436.
- 35 Pasparakis M, Alexopoulou L, Episkopou V, et al: Immune and inflammatory responses in TNF α -deficient mice: a critical requirement for TNF α in the formation of primary B cell follicles,

- follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med* 1996;184:1397–1411.
- 36 Saeki H, Moore AM, Brown MJ, et al: Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. *J Immunol* 1999;162:2472–2475.
 - 37 Boisleve F, Kerdine-Romer S, Rougier-Larzat N, et al: Nickel and DNCB Induce CCR7 expression on human dendritic cells through different signalling pathways: role of TNF- α and MAPK. *J Invest Dermatol* 2004;123:494–502.
 - 38 Wang B, Esche C, Mamelak A, et al: Cytokine knockouts in contact hypersensitivity research. *Cytokine Growth Factor Rev* 2003;14:381–389.
 - 39 Engeman TM, Gorbachev AV, Gladue RP, et al: Inhibition of functional T cell priming and contact hypersensitivity responses by treatment with anti-secondary lymphoid chemokine antibody during hapten sensitization. *J Immunol* 2000;164:5207–5214.
 - 40 Forster R, Schubel A, Breitfeld D, et al: CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 1999;99:23–33.
 - 41 Grabbe S, Steinbrink K, Steinert M, et al: Removal of the majority of epidermal Langerhans cells by topical or systemic steroid application enhances the effector phase of murine contact hypersensitivity. *J Immunol* 1995;155:4207–4217.
 - 42 Grabbe S, Schwarz T: Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. *Immunol Today* 1998;19:37–44.
 - 43 Nakano: Antigen-presenting cell function of epidermal cells activated by hapten application. *Br J Dermatol* 1998;138:786–794.
 - 44 Bennett CL, van Rijn E, Jung S, et al: Inducible ablation of mouse Langerhans cells diminishes but fails to abrogate contact hypersensitivity. *J Cell Biol* 2005;169:569–576.
 - 45 Valladeau J, Ravel O, Dezutter-Dambuyant C, et al: Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 2000;12:71–81.
 - 46 Pappenheimer AMJ, Harper AA, Moynihan M, et al: Diphtheria toxin and related proteins: effect of route of injection on toxicity and the determination of cytotoxicity for various cultured cells. *J Infect Dis* 1982;145:94–102.
 - 47 Kissenpfennig A, Henri S, Dubois B, et al: Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity* 2005;22:643–654.
 - 48 Valladeau J, Clair-Moninot V, Dezutter-Dambuyant C, et al: Identification of mouse langerin/CD207 in Langerhans cells and some dendritic cells of lymphoid tissues. *J Immunol* 2002;168:782–792.
 - 49 Kaplan DH, Jenison MC, Saeland S, et al: Epidermal Langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity* 2005;23:611–620.
 - 50 Enk A, Katz S: Early molecular events in the induction phase of contact sensitivity. *Proc Natl Acad Sci USA* 1992;89:1398–1402.
 - 51 Caughman SW, Li LJ, Degitz K: Characterization and functional analysis of interferon- γ -induced intercellular adhesion molecule-1 expression in human keratinocytes and A-431 cells. *J Invest Dermatol* 1990;94:22S–26S.
 - 52 Garioch JJ, Mackie RM, Campbell I, et al: Keratinocyte expression of intercellular adhesion molecule 1 (ICAM-1) correlated with infiltration of lymphocyte function associated antigen 1 (LFA-1) positive cells in evolving allergic contact dermatitis reactions. *Histopathology* 1991;19:351–354.
 - 53 Albanesi C, Cavani A, Girolomoni G: Interferon- γ -stimulated human keratinocytes express the genes necessary for the production of peptide-loaded MHC class II molecules. *J Invest Dermatol* 1998;110:138–142.
 - 54 Gaspari AA, Burns RP, Kondo S, et al: Characterization of the altered cutaneous reactivity of transgenic mice whose keratinocytes overexpress B7-1. *Clin Immunol Immunopathol* 1998;86: 259–270.
 - 55 Nasir A, Ferbel B, Salminen W, et al: Exaggerated and persistent cutaneous delayed-type hypersensitivity in transgenic mice whose epidermal keratinocytes constitutively express B7-1 antigen. *J Clin Invest* 1994;94:892–898.

- 56 Wakem P, Burns RP Jr, Ramirez F, et al: Allergens and irritants transcriptionally upregulate CD80 gene expression in human keratinocytes. *J Invest Dermatol* 2000;114:1085–1092.
- 57 Enk A, Katz S: Identification and induction of keratinocyte-derived IL-10. *J Immunol* 1992;149:92–95.
- 58 Burns R, Luzina I, Nasir A, et al: Keratinocyte-derived, CD80-mediated costimulation is associated with hapten-specific IgE production during contact hypersensitivity to TH1 haptens. *J Allergy Clin Immunol* 2005;115:383–390.
- 59 Masuda K, Katoh N, Soga F, et al: The role of interleukin-16 in murine contact hypersensitivity. *Clin Exp Immunol* 2005;140:213–219.
- 60 Yoshimoto T, Wang C-R, Yoneto T, et al: Role of IL-16 in delayed-type hypersensitivity reaction. *Blood* 2000;95:2869–2874.
- 61 Loser K, Mehling A, Loeser S, et al: Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. *Nat Med* 2006;12:1372–1379.
- 62 Mahnke K, Qian Y, Knop J, et al: Induction of CD4⁺/CD25⁺ regulatory T cells by targeting of antigens to immature dendritic cells. *Blood* 2003;101:4862–4869.
- 63 Theill LE, Boyle WJ, Penninger JM: RANK-L and RANK: T cells, bone loss, and mammalian evolution. *Annu Rev Immunol* 2002;20:795–823.
- 64 Bour H, Peyron E, Gaucherand M, et al: Major histocompatibility complex class I-restricted CD8⁺ T cells and class II-restricted CD4⁺ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene. *Eur J Immunol* 1995;25:3006–3010.
- 65 Martin S, Lappin MB, Kohler J, et al: Peptide immunization indicates that CD8⁺ T cells are the dominant effector cells in trinitrophenyl-specific contact hypersensitivity. *J Invest Dermatol* 2000;115:260–266.
- 66 Vocanson M, Hennino A, Cluzel-Tailhardat M, et al: CD8⁺ T cells are effector cells of contact dermatitis to common skin allergens in mice. *J Invest Dermatol* 2006;126:815–820.
- 67 Gocinski B, Tigelaar R: Roles of CD4⁺ and CD8⁺ T cells in murine contact sensitivity revealed by in vivo monoclonal antibody depletion. *J Immunol* 1990;144:4121–4128.
- 68 Kondo S, Beissert S, Wang B, et al: Hyporesponsiveness in contact hypersensitivity and irritant contact dermatitis in CD4 gene targeted mouse. *J Invest Dermatol* 1996;106:993–1000.
- 69 Saint-Mezard P, Chavagnac C, Vocanson M, et al: Deficient contact hypersensitivity reaction in CD4^{−/−} mice is because of impaired hapten-specific CD8⁺ T cell functions. *J Invest Dermatol* 2005;124:562–569.
- 70 Vocanson M, Hennino A, Chavagnac C, et al: Contribution of CD4⁺ and CD8⁺ T-cells in contact hypersensitivity and allergic contact dermatitis. *Expert Rev Clin Immunol* 2005;1:75–86.
- 71 Martin SF, Dudda JC, Delattre V, et al: Fas-mediated inhibition of CD4⁺ T cell priming results in dominance of type 1 CD8⁺ T cells in the immune response to the contact sensitizer trinitrophenyl. *J Immunol* 2004;173:3178–3185.
- 72 Watanabe H, Unger M, Tuvel B, et al: Review: contact hypersensitivity: the mechanism of immune responses and T cell balance. *J Interferon Cytokine Res* 2002;22:407–412.
- 73 Macatonia S, Hosken N, Litton M, et al: Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4⁺ T cells. *J Immunol* 1995;154:5071–5079.
- 74 Banerjee G, Damodaran A, Devi N, et al: Role of keratinocytes in antigen presentation and polarization of human T lymphocytes. *Scand J Immunol* 2004;59:385–394.
- 75 Riemann H, Schwarz A, Grabbe S, et al: Neutralization of IL-12 in vivo prevents induction of contact hypersensitivity and induces hapten-specific tolerance. *J Immunol* 1996;156:1799–1803.
- 76 Gorbachev AV, DiIulio NA, Fairchild RL: IL-12 augments CD8⁺ T cell development for contact hypersensitivity responses and circumvents anti-CD154 antibody-mediated inhibition. *J Immunol* 2001;167:156–162.
- 77 Xu H, DiIulio NA, Fairchild RL: T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon γ -producing (Tc1) effector CD8⁺ T cells and interleukin (IL) 4/IL-10-producing (Th2) negative regulatory CD4⁺ T cells. *J Exp Med* 1996;183:1001–1012.
- 78 Saulnier M, Huang S, Aguet M, et al: Role of interferon- γ in contact hypersensitivity assessed in interferon- γ receptor-deficient mice. *Toxicology* 1995;102:301–312.

- 79 Tsuji R, Kikuchi M, Askenase P: Possible involvement of C5/C5a in the efferent and elicitation phases of contact sensitivity. *J Immunol* 1996;156:4644–4450.
- 80 Tsuji RF, Geba GP, Wang Y, et al: Required early complement activation in contact sensitivity with generation of local C5-dependent chemotactic activity, and late T cell interferon γ : a possible initiating role of B cells. *J Exp Med* 1997;186:1015–1026.
- 81 Tsuji RF, Kawikova I, Ramabhadran R, et al: Early local generation of C5a initiates the elicitation of contact sensitivity by leading to early T cell recruitment. *J Immunol* 2000;165:1588–1598.
- 82 Barrington R, Zhang M, Fischer M, et al: The role of complement in inflammation and adaptive immunity. *Immunol Rev* 2001;180:5–15.
- 83 Yancey KB, Hammer CH, Harvath L, et al: Studies of human C5a as a mediator of inflammation in normal human skin. *J Clin Invest* 1985;75:486–495.
- 84 Stall AM, Wells SM, Lam K-P: B-1 Cells: unique origins and functions. *Semin Immunol* 1996; 8:45–59.
- 85 Tsuji RF, Szczepanik M, Kawikova I, et al: B cell-dependent T cell responses: IgM antibodies are required to elicit contact sensitivity. *J Exp Med* 2002;196:1277–1290.
- 86 Askenase PW, Szczepanik M, Itakura A, et al: Extravascular T-cell recruitment requires initiation begun by $V\alpha 14+$ NKT cells and B-1 B cells. *Trends Immunol* 2004;25:441–449.
- 87 Mercer JC, Ragin MJ, August A: Natural killer T cells: rapid responders controlling immunity and disease. *Int J Biochem Cell Biol* 2005;37:1337–1343.
- 88 Hansen DS, Schofield L: Regulation of immunity and pathogenesis in infectious diseases by CD1d-restricted NKT cells. *Int J Parasitol* 2004;34:15–25.
- 89 Kawano T, Cui J, Koezuka Y, et al: Natural killer-like nonspecific tumor cell lysis mediated by specific ligand-activated $V\alpha 14$ NKT cells. *Proc Natl Acad Sci USA* 1998;95:5690–5693.
- 90 Akbari O, Faul JL, Hoyte EG, et al: CD4+ invariant T-cell-receptor+ natural killer T cells in bronchial asthma. *N Engl J Med* 2006;354:1117–1129.
- 91 Hong S, Wilson MT, Serizawa I, et al: The natural killer T-cell ligand α -galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat Med* 2001;7:1052–1056.
- 92 Zeng D, Liu Y, Sidobre S, et al: Activation of natural killer T cells in NZB/W mice induces Th1-type immune responses exacerbating lupus. *J Clin Invest* 2003;112:1211–1222.
- 93 Kakimi K, Guidotti LG, Koezuka Y, et al: Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *J Exp Med* 2000;192:921–930.
- 94 Campos RA, Szczepanik M, Itakura A, et al: Cutaneous immunization rapidly activates liver invariant $V\alpha 14$ NKT cells stimulating B-1 B cells to initiate T cell recruitment for elicitation of contact sensitivity. *J Exp Med* 2003;198:1785–1796.
- 95 Ptak W, Herzog W, Askenase P: Delayed-type hypersensitivity initiation by early-acting cells that are antigen mismatched or MHC incompatible with late-acting, delayed-type hypersensitivity effector T cells. *J Immunol* 1991;146:469–475.
- 96 Campos RA, Szczepanik M, Itakura A, et al: Interleukin-4-dependent innate collaboration between iNKT cells and B-1 B cells controls adaptative contact sensitivity. *Immunology* 2006;117: 536–547.
- 97 Campos RA, Szczepanik M, Lisbonne M, et al: Invariant NKT cells rapidly activated via immunization with diverse contact antigens collaborate in vitro with B-1 cells to initiate contact sensitivity. *J Immunol* 2006;177:3686–3694.
- 98 Askenase PW, Itakura A, Leite-de-Moraes MC, et al: TLR-dependent IL-4 production by invariant $V\alpha 14+J\alpha 18+$ NKT cells to initiate contact sensitivity in vivo. *J Immunol* 2005;175:6390–6401.
- 99 Kondo S, McKenzie RC, Sauder DN: Interleukin-10 inhibits the elicitation phase of allergic contact hypersensitivity. *J Invest Dermatol* 1994;103:811–814.
- 100 Maguire HCJ, Ketcha KA, Lattime EC: Neutralizing anti-IL-10 antibody upregulates the induction and elicitation of contact hypersensitivity. *J Interferon Cytokine Res* 1997;17:763–768.
- 101 Berg D, Leach M, Kuhn R, et al: Interleukin 10 but not interleukin 4 is a natural suppressant of cutaneous inflammatory responses. *J Exp Med* 1995;182:99–108.
- 102 Desvignes C, Etchart N, Kehren J, et al: Oral administration of hapten inhibits in vivo induction of specific cytotoxic CD8+ T cells mediating tissue inflammation: a role for regulatory CD4+ T cells. *J Immunol* 2000;164:2515–2522.

- 103 Sakaguchi S, Sakaguchi N, Asano M, et al: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995;155:1151–1164.
- 104 Hori S, Nomura T, Sakaguchi S: Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057–1061.
- 105 Liu H, Leung BP: CD4+CD25+ regulatory T cells in health and disease. *Clin Exp Pharmacol Physiol* 2006;33:519–524.
- 106 Shevach EM: CD4+CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002;2:389–400.
- 107 Colantonio L, Iellem A, Sinigaglia F, et al: Skin-homing CLA+ T cells and regulatory CD25+ T cells represent major subsets of human peripheral blood memory T cells migrating in response to CCL1/I-309. *Eur J Immunol* 2002;32:3506–3514.
- 108 Cavani A, Nasorri F, Ottaviani C, et al: Human CD25+ regulatory T cells maintain immune tolerance to nickel in healthy, nonallergic individuals. *J Immunol* 2003;171:5760–5768.
- 109 Ring S, Schäfer SC, Mahnke K, et al: CD4+ CD25+ regulatory T cells suppress contact hypersensitivity reactions by blocking influx of effector T cells into inflamed tissue. *Eur J Dermatol* 2006;36:2981–2992.
- 110 Cavani A, Nasorri F, Prezzi C, et al: Human CD4+ T Lymphocytes with remarkable regulatory functions on dendritic cells and nickel-specific Th1 immune responses. *J Invest Dermatol* 2000;114:295–302.
- 111 Roncarolo MG, Bacchetta R, Bordignon C, et al: Type 1 T regulatory cells. *Immunol Rev* 2001;182:68–79.
- 112 Girolomoni G, Gisondi P, Ottaviani C, et al: Immunoregulation of allergic contact dermatitis. *J Dermatol* 2004;31:264–270.
- 113 Dieckmann D, Bruett CH, Ploettner H, et al: Human CD4+CD25+ regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. *J Exp Med* 2002;196:247–253.
- 114 O’Leary JG, Goodarzi M, Drayton DL, et al: T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol* 2006;7:507–516.

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Immune Privilege and the Skin

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Abstract

This chapter summarizes the evidence that defined compartments of the hair follicle (HF) and nail epithelium maintain an area of relative immune privilege (IP). HF and nail IP is chiefly characterized by absent or very low level of expression of major histocompatibility complex class Ia antigens, complemented by a number of factors, such as the local production of potent immunosuppressive agents, dysfunction of professional antigen-presenting cells and inhibition of natural killer cell activities. In the hair bulb, IP is seen only in the anagen stage of HF cycling, while the nail apparatus continuously maintains an IP site in its proximal nail matrix, since the nail apparatus does not cycle. Possibly, the (non-cycling) bulge area of human scalp HFs also enjoys some relative, stably maintained IP, even though it is not as pronounced as the IP of the anagen hair bulb. A collapse of HF and nail IP likely plays a key role in the pathogenesis of one of the most common organ-specific autoimmune diseases, alopecia areata. Therefore, the therapeutic restoration of IP collapse promises to be a particularly effective future strategy for the treatment of alopecia areata.

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What Is Immune Privilege?

The concept of immune privilege (IP) was derived from the acceptance of allogeneic tumor implants in defined anatomical locations, such as the eye and the brain [1]. Because these transplanted tissues survived longer than anticipated [2], the term immunological privilege was primarily coined in relation to transplant (for example corneal allograft) survival in these sites [3]. Thus, originally, the term IP served to illustrate that a given tissue environment that hosts an allotransplant awards the transplanted cells relative protection from rejection by the host immune system [4, 6].

However, the definition of IP was extended to tissue sites where the local establishment of an immunosuppressive/tolerogenic environment exerts biologically important functions, such as in the fetomaternal placental unit, where IP is vital for avoiding fetal rejection [9, 10], and in the eye, where ocular IP is indispensable for normal eye function [8]. Correspondingly, the collapse of eye IP may result in autoimmune uveitis, that is potentially blinding ocular inflammatory diseases. Today, the term IP has become associated with a small list of defined anatomical compartments that are protected by IP from excessive inflammatory activity, such as the anterior chamber of the eye, the fetotrophoblast, the testis, the central nervous system behind the blood-brain barrier, the anagen hair follicle (HF) epithelium [11], the proximal nail matrix (PNM) [12] and the hamster cheek pouch [4–8].

Corresponding autoimmune diseases in which IP collapse are now appreciated to play a major role and include multiple sclerosis [13], mumps orchitis [14] and autoimmune chronic active hepatitis [14–16].

IP is generally established and maintained by [5, 8–10, 17]:

- Downregulation or absence of classical major histocompatibility complex (MHC) class I expression, which sequesters (auto)antigens in tissue sites and hinders their presentation to CD8+ T cells with a matching T cell receptor.
- Local production of potent immunosuppressants such as TGF- β 1, IL-10 and α -melanocyte-stimulating hormone (MSH).
- Functional impairment of antigen-presenting cells, for example by down-regulation of MHC class II expression.
- Absence of lymphatics.
- Establishment of extracellular matrix barriers to hinder immune cell trafficking, such as blood-retinal barrier and blood-brain barrier.
- Expression of nonclassical MHC class I molecules (such as the MHC class Ib molecules HLA-G in humans and Qa-2 in mice).
- Expression of Fas ligand (FasL) in order to delete autoreactive, Fas-expressing T cell mechanisms.

In addition to these mechanisms of IP maintenance, new conditions and factors have been explored that may also contribute to the generation and maintenance of relative IP. For example, the number of allogenic concepts in female mice treated with indoleamine 2,3-dioxygenase (IDO) inhibitor was reduced significantly compared to control mice. IDO is a tryptophan-catabolizing enzyme which catabolizes tryptophan into N-formylkynurenine which is converted into 3-hydroxyanthranilic acid. Tryptophan is an essential amino acid, and is required for normal T cell proliferation. The catabolism of tryptophan by IDO expression in tissues or antigen-presenting cells locally starves T cells and limits their proliferation in response to antigen. Thus, IP may be maintained as

a result of the enzymatic activity of IDO [9, 18], which downregulates T cell function by tryptophan depletion [19]. In addition, murine regulatory T cells can condition dendritic cells to express IDO functional activity and thus to exercise immunosuppressive functions in the context of IP. Inhibition of complement activation [20] and a placenta-specific, β 2-microglobulin-dependent process linked to MHC class I antigen presentation [21] are other examples for additional, newly recognized mechanisms that favor maternal immunotolerance of pregnancy; uterine natural killer (NK) cells may also be involved [10].

IP is a relative, not an absolute, state, and only some of these mechanisms, whose composition and relative importance vary between tissue sites, may be present in a recognized IP compartment [7, 22]. For example, even though fetal cells expressing paternal antigens can leave the fetomaternal placental unit and do evoke a maternal response [23], and even though paternal DNA is found in the lesions of one of the classical dermatoses associated with pregnancy, fetal rejection is usually not a consequence. This suggests that the critical issue is whether, and to what extent, a state of tolerance to the alloantigen-bearing cells is established and how well immunotolerance is maintained throughout the persistence of these antigens.

IP in the Skin and Its Appendages

On this background, the skin and its appendages deserve careful scrutiny. The skin is continuously facing exposure to various kinds of antigens, including potentially deleterious ones such as bacteria, viruses and fungi, as well as a large variety of chemical substances and allergens, to which the immune system has to mount desirable immune responses, while undesirable ones must be suppressed. Thus, the skin needs to strike a delicate balance between efficient defense against infection, ‘danger alert’, maintenance of immune tolerance against autoantigens and avoidance of autoaggressive, inflammatory tissue destruction. Therefore, the skin is continuously challenged with having to maintain a fine balance between proper immune defenses, the limitation of damaging inflammation, specifically the avoidance of deleterious autoimmunity.

In this context, it is noteworthy that at least two regions of the skin epithelium are now recognized to meet key characteristics of IP sites, that is the epithelium of the two major skin appendages: the anagen hair bulb and the PNM [11]. In addition, it is currently being explored whether the stem cell-rich bulge region of the HF also meets IP criteria [24]. Unfortunately, however, our understanding of skin IP is still very patchy and unsatisfactory, since these immunoprivileged sites in mammalian skin have so far been largely ignored by mainstream IP research. Therefore, one can at best sketch outlines of the potential

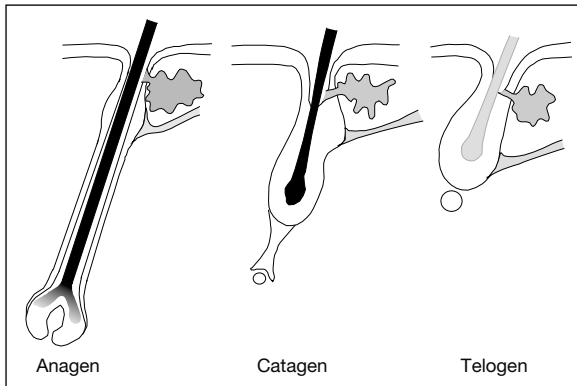


Fig. 1. Hair cycle of human scalp HFs. As all HFs – one of the defining feature of the mammalian species – normal human scalp HFs show striking, cyclic organ transformation and remodeling events. This so-called hair cycle displays three stages: growth (anagen), regression (catagen) and rest (telogen).

significance of skin IP, drawing primarily upon the limited available information that has been published so far on the IP of HF and nail.

HFs are one of the defining features of mammals. These skin appendages generate pigmented keratin fibers (hair shafts) during their growth phase (called anagen). Hair follicles are prototypic neuroectodermal-mesodermal interaction systems, since hair follicles undergo continuous remodeling events (called the hair cycle), which result from complex bidirectional interactions between the HF epithelium and its specialized, inductive mesenchyme. Thus, the generation of pigmented hair shafts during anagen requires precisely coordinated interactions between HF keratinocytes, melanocytes and fibroblasts (fig. 1) [25–27]. Since melanocyte-associated antigens frequently are the target of cutaneous autoimmunity (for example in the context of vitiligo, halo nevi or malignant melanoma regression) [28–31], it is reasonable to expect that HF melanocytes and their pigmentary activities may also be targeted by autoimmune responses. Indeed, it is now believed that HF melanocyte autoantigens play a key role as potential immune targets in one of the most frequent autoimmune diseases of man, alopecia areata (AA), where the presence of autoantibodies against melanocyte-associated autoantibodies has long been appreciated, for example [11, 32, 33].

Intriguingly, more than three decades ago, Billingham had already noted that the anagen hair bulb, the factory for pigmented hair shafts [26, 27], provides a special milieu that allows transplanted allogeneic melanocytes to escape

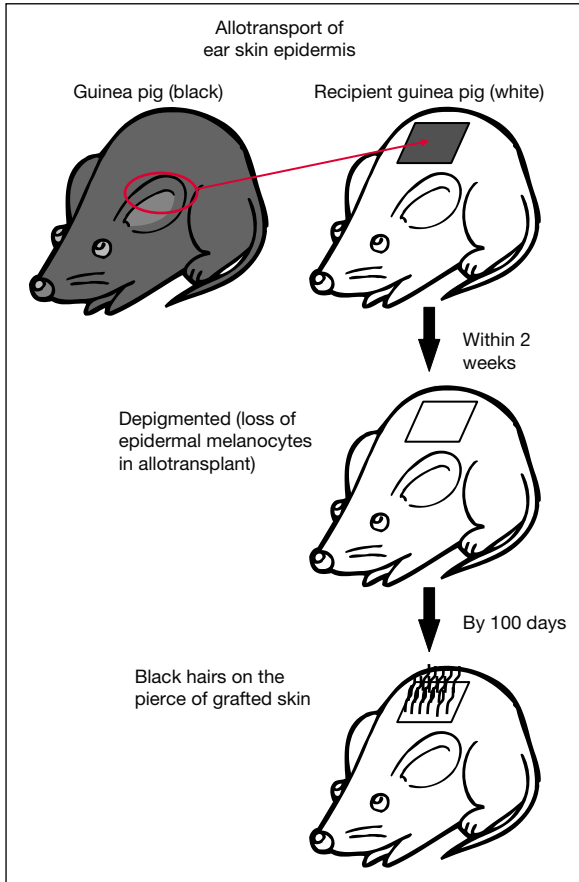


Fig. 2. Black ear skin epidermis transplanted onto skin beds of genetically incompatible white guinea pigs immediately lost its pigmentation within 2 weeks as a sign of the rejection of foreign melanocytes. However, black hair shafts began to pierce the (now white) epidermis by 100 days. This result indicates that melanocytes could escape from rejection of immune system because of the immunotolerated milieu of the anagen hair bulb (modified from Paus et al. [11]).

from recognition and rejection by the host immune system. Billingham noted that black ear skin epidermis transplanted onto skin beds of genetically incompatible white guinea pigs immediately lost its pigmentation as a sign of the rejection of foreign melanocytes. However, black hair shafts soon thereafter began to pierce the (now white) epidermis, indicating that at least some donor melanocytes had survived in the host hair bulbs and resumed their transfer of melanosomes to precortical hair matrix keratinocytes (fig. 2) [34, 35].

Despite these exciting early findings pointing to the anagen hair bulb as a site of IP (which, however, remain to be reproduced by independent investigators and in additional experimental models), the concept of HF IP still awaits systematic exploration by mainstream immunology. This has become even more urgent, since the human PNM [12] and the HF bulge [24] have recently also surfaced as strong candidates as sites of intracutaneous IP.

Anagen-Dependent Immunosuppression

To fully appreciate HF IP, it is necessary to appreciate it in front of a tapestry of hair cycle-dependent skin immunity changes – which, again, is still largely ignored by mainstream immunology. One needs to recall that the HF continuously undergoes cyclic tissue remodeling events which result in three hair cycle stages: massive epithelial cell proliferation and terminal differentiation (anagen), followed by rapid, apoptosis-driven organ involution (catagen) and relative resting (telogen). In mice, highly synchronized HF cycling can be induced by depilation of hair shafts on the back of mice with all HFs in telogen [36]. Intriguingly, HF cycling in mice is associated with significant fluctuations both in the skin immune status, and in the HF immune system.

Most notably, delayed-type hypersensitivity reaction (contact hypersensitivity) and photosensitivity are influenced by hair cycling in C57BL/6 and BALB/c mice [37, 38]. The sensitization phase of experimentally induced contact hypersensitivity to picryl chloride strictly depends on the predominant hair cycle stage of murine skin. If administered via back skin with all HF in telogen, challenge of the earlobe with the hapten induces vigorous ear swelling, whereas the response is essentially abrogated if sensitization is performed through back skin with all HF in synchronized, depilation-induced anagen (fig. 3). This anagen-dependent suppression of type IV skin immune responses appears to be associated not only with local intracutaneous, but also with systemic immunosuppression [37]. Although the underlying mechanisms remain to be elucidated (for example anagen-associated intracutaneous production of immunosuppressive cytokine neuropeptides and/or cytokines, such as TGF- β [39, 40]), it is reasonable to speculate that this striking hair cycle dependence of the skin immune status in mice is somehow associated with the IP of anagen HFs that is further described below.

The Anagen Hair Bulb as an IP Site

More than a decade after Billingham's discovery, Harrist et al. [41] reported the distribution of MHC antigens in normal human skin including

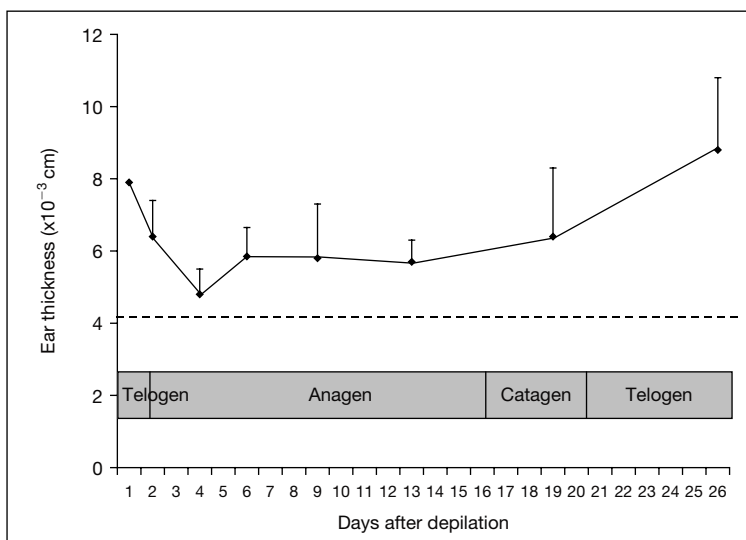


Fig. 3. Delayed-type hypersensitivity reaction and photosensitivity are influenced by HF cycling in C57BL/6 and BALB/c mice. If administered via back skin with all HF in telogen, challenge of the earlobe with the hapten induces vigorous ear swelling, whereas the response is abrogated if sensitization is performed through back skin with all HF in synchronized, depilation-induced anagen (modified from Paus et al. [36] and Hofmann et al. [37]).

human terminal HFs. These authors found that MHC class I molecules are present on the surface of epidermal basal and spinous layer keratinocytes, and on the outer root sheath (ORS) epithelium in the infundibulum of the HF. In contrast, dermal papilla, proximal ORS and inner root sheath (IRS) showed negative MHC class I expression. Ia-like, antigen-positive dendritic cells are also observed rarely in deep portion (around the proximal HFs). On the other hand, distal ORS shows strong positive expression of MHC class I and many Ia-like, antigen-positive dendritic cells. This striking downregulation of MHC class I expression in the proximal epithelium of anagen hair bulbs was confirmed to exist in human [42], rat [43] and mouse HFs [44], and then reanalyzed in greater detail in stage VI human anagen scalp HFs (fig. 4a) [45]. In these studies, the question whether the human HF bulge in the area of stem cells is relatively immunoprivileged too has been left out so far. The most recent study may reveal that the bulge region of the anagen HF also shows the character of a relative site of IP [23], because the expression of MHC class I is very low or absent.

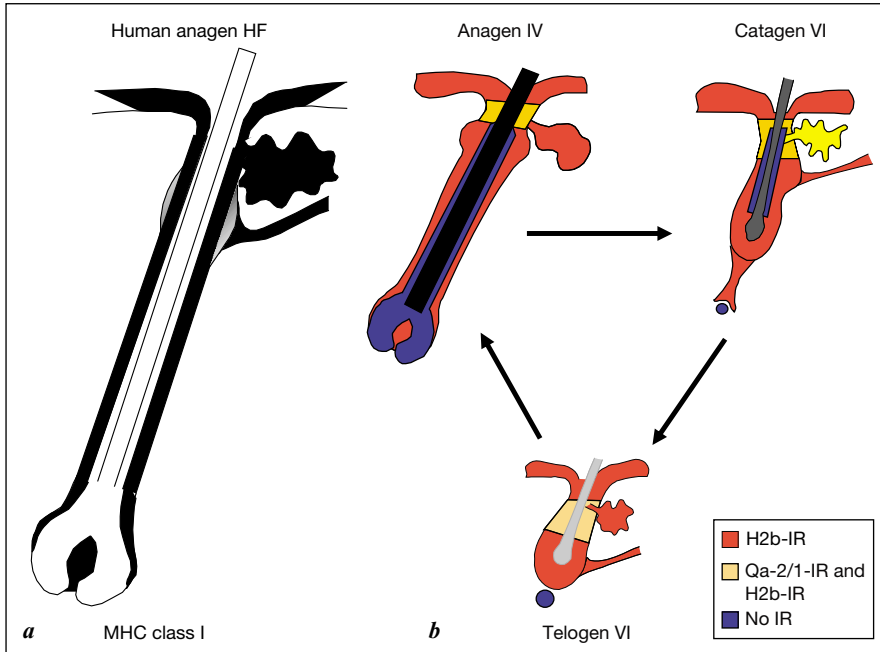


Fig. 4. *a* Expression of MHC class I antigens in human HF. The expression of MHC class Ia is downregulated in proximal ORS. Dermal papilla shows strong expression of MHC class I. Bulge area has much lower expression and often apparently absent MHC class I IR (modified from Paus et al. [44]). *b* The intrafollicular expression pattern of classical and non-classical MHC class I during the murine hair cycle. Classical MHC class I expression changes during the murine hair cycle. The representative character of HF IP downregulation of MHC class I is only observed during the anagen phase. Nonclassical MHC class Ib molecule like Qa-2 is continuously expressed in the area of infundibulum (modified from Westgate et al. [43]).

The claim that defined epithelial compartments of the HF have established IP is not only based on their MHC class I negativity, but also on other observations that all point to a site with relative IP [11]:

- In human stage VI anagen scalp HFs, CD4+ T cells are observed only extremely rarely, and CD8+ T cells and CD1a+ cells are almost always absent (fig. 5). If present, these cells are predominantly distributed in the distal HF epithelium [45].
- In the proximal portion of the HF, there is a sharply reduced number of apparently nonfunctional, MHC class II antigen-negative Langerhans cells, [45] compared with the distal part of HFs (upper portion of HFs).

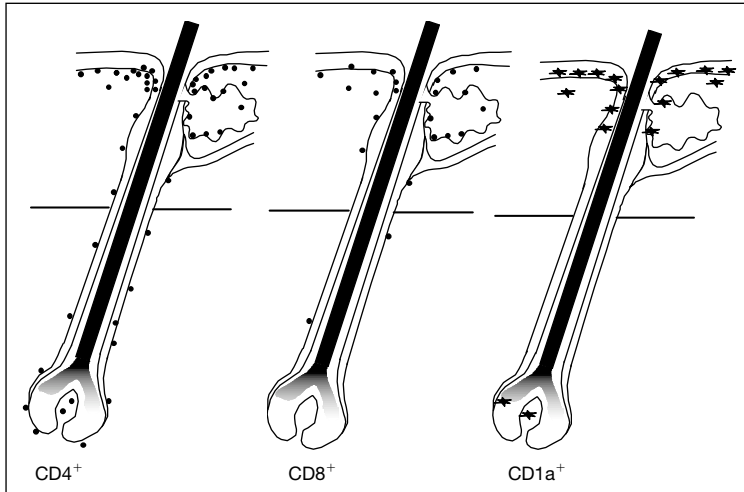


Fig. 5. Distribution of peri- and intrafollicular immune cells. The distribution of immune cell in and around human HF differs from that in and below the interfollicular epidermis. Around the human anagen hair bulb, CD4⁺, CD8⁺ and CD1a⁺ cells are only rarely found. Intrafollicular Langerhans cells appear to be functionally impaired because they do not express MHC class II antigens.

- Contrary to the ORS distal from the infundibulum of the sebaceous gland, the anagen hair bulb is almost devoid of intraepithelial T cells; and in mice, $\gamma\delta$ TCR⁺ lymphocytes are not detected below the bulge region [46]. Anagen hair bulbs show a greatly reduced number of antigen-presenting cells (CD1a⁺ cells or ultrastructurally identified Langerhans cells), which appear to be functionally impaired because they do not express MHC class II antigens [45]. Unlike the ORS distal from the infundibulum of the sebaceous gland, the anagen hair bulb is almost devoid of intraepithelial T cells; in mice, no TCR⁺ lymphocytes are found below the bulge region [46]. Human anagen VI scalp HFs show CD4⁺ T cells extremely rarely, and a CD8⁺ lymphocyte is almost never caught trafficking through the proximal follicle epithelium [45].
- Like the other well-defined immune-privileged tissues, the hair bulb is characterized by the absence of lymphatic drainage pathway and the presence of a special extracellular matrix barrier around the HF, both of these conditions may contribute to hinder immune cell trafficking [43, 47].
- Importantly, anagen hair bulbs in mice and man express potent immunosuppressants such as TGF- β 1 [39, 48], ACTH [49, 50] and α -MSH [50–54].

In addition, gene profiling data [55, 56] have raised the possibility that not only the anagen hair bulb, but also the bulge may display an immunoprivilege, even though it may be less capable than the anagen hair bulb. Most recent immunohistological analyses confirm this concept by showing that MHC class Ia and II protein expressions are downregulated in the bulge region of human HFs, whereas both CD200 receptors and locally generated potent immunosuppressants like TGF- β 1 and α -MSH are upregulated [24].

Classical and Nonclassical MHC Class I Expression in Murine HF

The gene loci of the MHC region are grouped into three classes (I–III). MHC class I is separated into classical (MHC Ia) and nonclassical (MHC Ib). Principal MHC class I gene, called classical MHC class I gene, is subdivided into HLA-A, HLA-B and HLA-C in human and Qa, Tla and M in mice. HLA-E, HLA-F, HLA-G and HLA-H genes also encode nonclassical MHC class I proteins, also called class Ib. MHC class I is basically expressed on all nucleated cells [57]. However, several organs or tissue compartments with an established IP are characterized by a lack of classical MHC class I expression [11].

This characteristic feature of anagen HF IP, that is negativity for classical MHC class I expression, is found on the proximal ORS and hair matrix. The details of classical MHC class I expression on HF have been investigated, using the murine hair cycle as a model. Immunoreactivity (IR) for classical MHC class I antigen in the mouse HF is strikingly dependent on specific hair cycle and exhibits significant differences between various anatomical follicle compartments (fig. 4b) [44]. The cycle dependency of murine HF MHC class I antigen can be illustrated in experiments in which anagen is induced in the back skin of the mouse (for example C57BL/6) by depilation of the HFs in telogen (relative resting phase of the cycle) [36]. During the entire hair cycle, the distal part of the HF continuously shows strong classical MHC class I IR. In the telogen stage, the entire HF compartments have a strong classical MHC class I IR except for dermal papilla. Shortly after anagen induction, this IR pattern changes. In anagen stages II–III, anagen hair matrix and dermal papilla show negative classical MHC class I IR. In anagen stage VI, the IRS, in addition to the hair matrix, has no classical MHC class I IR, but the MHC class Ia IR reappears in dermal papilla. In spontaneously developed catagen HFs, only the slowly receding IRS keratinocytes and dermal papilla remain MHC class Ia negative (which may reflect an artefact due to antigen masking [44]).

In the human fetomaternal placental unit (fetotrophoblast), nonclassical MHC class I molecule HLA-G impairs specific cytolytic T cell functions, in

addition to exerting its well-established inhibitory functions on cell lysis by NK cells [58]. In fact, expression of a mouse nonclassical MHC class I equivalent, Qa-2, could be detected in the periinfundibular region of the murine ORS throughout the entire hair cycle [44] (fig. 4b). The hair cycle-independent expression of Qa-2 raises the possibility that these MHC class Ib molecules are involved in the regulation of follicular IP, rather than in the intriguing, nonspecific antiinfection defenses of the HF immune system [44, 59], and encourages the hypothesis that MHC class Ib expression may play a similar T and NK cell-inhibitory role in the context of the HF IP as it does in the fetomaternal placental unit [58].

MHC Class I Expression and Human HF

The striking downregulation of MHC class Ia expression in the proximal epithelium of anagen hair bulbs is also seen in rat [43] and human [42] HFs. Although corresponding expression changes during whole human hair cycle remain to be elucidated, MHC class I expression has been studied in greater detail in human anagen VI scalp HFs [45, 60]. Here, MHC class I IR was found on all interfollicular cutaneous cells. In comparison with the epidermis and the distal ORS, the isthmus region of the ORS showed substantially reduced MHC class I IR, and a striking restriction of MHC class I IR to basal keratinocytes. The proximal ORS, IRS, all hair bulb keratinocytes and most of the dermal papilla displayed no detectable MHC class I IR. Sporadically, single MHC class I-positive cells were found within the dermal papilla. Furthermore, the bulge area of human anagen VI scalp HFs also shows a sharply reduced expression of MHC class I IR, implying that the bulge area is a second site of IP within the HF [24]. In contrast, perifollicular fibroblasts and immune cells of the perifollicular CTS were MHC class I positive.

HF Expression of MHC Class I Pathway-Associated Molecules

Antigen presentation in the context for the MHC class I molecules to CD8+ cytotoxic T cells requires the ATP-dependent transporter in antigen presentation (TAP), consisting of the subunits TAP1 and TAP2 [61]. TAP1 and TAP2 genes are in the MHC class II region that must be expressed for MHC class I molecules to be assembled efficiently [62]. Peptides generated in the cytosol by the proteasome are translocated by TAP1 and TAP2 into rough endoplasmic reticulum (RER) by a process that requires the hydrolysis of ATP. Within the RER

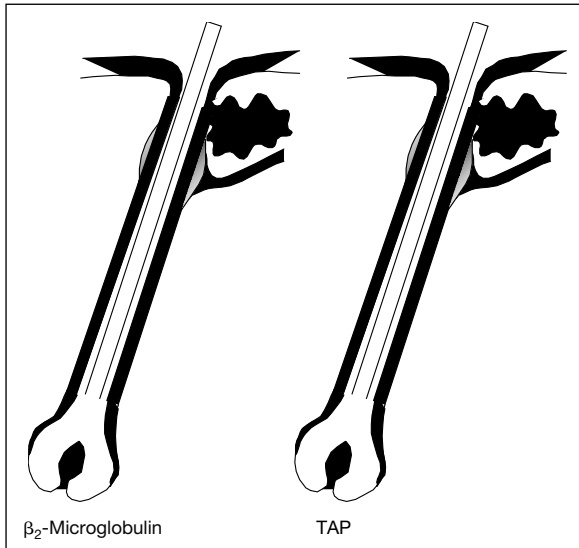


Fig. 6. The expression of MHC class I pathway molecules on the murine anagen HF. The expression of the MHC class I pathway molecules TAP and β_2 -microglobulin is mirroring the IR of HLA-A/HLA-B/HLA-C molecules.

membrane, newly synthesized class I α -chain associates with calnexin until β_2 -microglobulin binds to the α -chain. The class I α -chain/ β_2 -microglobulin heterodimer then binds to calreticulin and the TAP-associated protein tapasin. When a peptide delivered by TAP is bound to the class I molecules, folding of MHC class I is complete and it is released from the RER and transported through the Golgi complex to the surface.

The expression of these MHC class I pathway molecules, such as TAP and β_2 -microglobulin was analyzed in human and murine HFs. The follicular expression of these MHC class I pathway molecules was detected by immunohistology. β_2 -microglobulin and TAP2 IR mirrored the low or absent expression level of HLA-A/HLA-B/HLA-C molecules in the proximal ORS and hair matrix of anagen hair bulbs compared to distal ORS and epidermis in the normal human scalp skin sections (fig. 6) [60]. Therefore, this immunoprivileged region of the skin epithelium is characterized by a downregulation not only of HLA-A/HLA-B/HLA-C, but also of major MHC class I pathway molecules. This mirrors the situation in murine HFs in vivo and underscores the relative defect of these follicular tissue compartments in the presentation of self-peptides

to CD8+ T cells [63]. Although the fluctuation of MHC class I pathway molecule expression during the entire human and murine hair cycle still awaits comprehensive assessment, currently available information already suggests that these MHC class I pathway-associated molecules are intimately involved in maintaining HF IP.

Downregulation of MHC Class I and NK Cells in HF IP

Normally, NK cells attack autologous cells (such as virally infected or malignantly transformed cells) with absent or low MHC class I expression. Surprisingly, however, only very few perifollicular NK cells are ever found around healthy human anagen HFs [45], and the concept of HF IP needs to provide reasonable explanations for the striking, seemingly contradictory absence of intrafollicular MHC class Ia and perifollicular NK cells.

Normally, NK cells express inhibitory receptors, such as killer cell immunoglobulin-like receptors (KIR) and heterodimer CD94/NKG2A [64], and if target cells express MHC class I molecules, NK cell activation can be prevented through interaction with KIR by phosphorylation of immunoreceptor tyrosine inhibitory motif followed by binding to phosphatases including SHP-1 and SHP-2 [64, 65]. Target cells lacking MHC class I expression (for example after viral infection or malignant transformation) do fail to inhibit NK cells, and NKG2D expressed on NK cells recognizes the MHC class I chain-related A gene (MICA) on target cells [66, 67].

In order to better understand NK cell activity in the context of HF IP, we compared normal anagen HF with HFs from patients with AA – an organ-specific, cell-mediated autoimmune disease thought to result from a collapse of HF IP [11, 32, 33]. In fact, recent evidence from our laboratories suggests that lesional skin in AA patients shows both, a marked upregulation of NK cell numbers and a defect in keeping perifollicular NK cell activity in check, while peripheral blood NK cells in AA patients may be constitutively more sensitive to activating stimuli. The normal anagen HFs may escape from NK cell attack by combining active suppression of NK cells (for example by MIF and KIR upregulation) with reducing the chance of NK cells to receive stimulatory signals (for example by downregulation of NKG2D expression on NK cells and of its ligand, MICA, on the potential target, HF epithelium). Patients with AA show striking, previously unknown, intracutaneous and systemic defects in NK cell containment. These recently revealed defects must now be taken into account in AA pathogenesis, and in the development of more effective treatment scenarios for AA and related autoimmune diseases that are also characterized by IP collapse [68].

Immunosuppressants and HF IP

Pro-opiomelanocortin (POMC)-derived peptides include ACTH, MSH and β -endorphin. POMC gene transcription and translation are hair cycle dependent in the skin of C57BL/6 mice and increase significantly in the active growth stage of the hair cycle (anagen) [69]. These molecules have immunosuppressive effects so that each peptide may contribute to the immunoprivileged milieu in HF. On normal skin, ACTH IR is localized on the ORS cells of anagen HFs that are not detected in the epidermis and dermis of corporal skin [49]. α -MSH is detected in keratinocytes of the ORS and hair matrix during anagen [51]. The concentration of ACTH significantly increases during depilation-induced anagen measured by radioimmunoassay [40]. Together with the findings that the epithelium of human anagen VI HFs in scalp skin also prominently expresses α -MSH and ACTH, that ACTH stimulates intrafollicular cortisol generation [53], and that α -MSH is a potent general immunosuppressant [70], these results indicate that both melanocortins may contribute to the maintenance of IP milieu in the anagen stage.

TGF- β is one of the most potent immunosuppressive growth factors described so far [71]. Interestingly, TGF- β 1 IR is prominently expressed in the HF epithelium of mice and man, and is strongest during late anagen and the onset of catagen in cells of the ORS and epithelial strand [48]. TGF- β receptor II-positive cells were also found in the proximal and central region of the ORS during the late anagen- and catagen-induced hair growth in mink [39, 48, 72]. Therefore, together with ACTH, α -MSH and cortisol, TGF- β 1 likely constitutes an integral part of an entire system of intrafollicularly generated, secreted 'IP maintenance/protective factors' [11], which remains to be dissected comprehensively.

What Is the Function of HF IP?

While it is now clear that IP is indispensable for normal eye function to avoid inflammatory reactions, and that IP in the fetomaternal placental unit is vital for the suppression of fetal rejection [5, 6, 8–10, 72], it still remains to be satisfactorily demonstrated by convincing functional evidence what exactly HF IP might be good for. With regard to ocular IP, Niederkorn [8, 73] concludes that the existence of ocular IP is necessary for the normal function of the visual axis, whose preservation requires that ocular inflammation is very stringently regulated and innocent bystander immune damage that may result in irreversible eye damage is scrupulously avoided. Along the same lines, establishment of an IP milieu in the HF may be needed to secure a safe environment for

HF cycling, the process of which depends on a constitutively active safeguarding system to protect it against immune injury.

In this context it is noteworthy that, apart from epidermal melanocytes (in vitiligo), thyroid epithelium (in autoimmune thyroiditis) and the synovium (in rheumatoid or psoriatic arthritis) [7, 22], the HF represents one of the most frequent targets of immune-mediated disease, resulting in the development of AA, or even in permanent disease (due to immune destruction of epithelial HF stem cells) as it is seen in the scarring alopecia associated with lichen planopilaris, lupus erythematosus, scleroderma and folliculitis decalvans [27, 74]. Therefore, one reasonable general hypothesis is that HF IP serves to reduce the chances of autoimmune HF damage [11].

Skin melanocytes are prone to be the target of immune-mediated injury (for example in vitiligo, halo nevi, regressing malignant melanoma and during immunotherapy of metastasizing melanoma). Notably, in AA lesions, the characteristic inflammatory cell attack on lesional HFs almost exclusively targets anagen hair bulbs which are in the process of active pigment production (that is anagen III–VI HFs). In addition, recovering HFs in AA patients typically generate white hair shafts [44, 74]. Thus, a more specific hypothetical function of HF IP is to sequester melanocyte-/melanogenesis-associated autoantigens from immune recognition and to protect the hair bulb from potentially deleterious autoaggressive immune responses. Immunogenetically distinct individuals may differ in their relative level of protection from, and relative risk of, anti-HF autoimmunity. In case of constitutively insufficient IP capacity or functional collapse of the HF IP, this would result in a greatly enhanced risk of autoimmune attack on the follicle [11, 44, 76].

It is interesting to note that during neonatal HF morphogenesis in mice, in contrast to the epidermis, distal follicular keratinocytes begin to express MHC class I only at the late stage of development when almost all skin cells already express MHC class I molecules, paralleling the timing of maturation of the skin immune system [55, 59, 76]. This observation raises the question if IP is also needed for the proper development of HF epithelium. Perhaps, IP needs to be established by the time when the full complement of immunocytes (intraepithelial T cells and Langerhans cells), immunoregulatory surface molecules, secreted immunomodulators and HF melanocytes has been assembled and expressed in their designated locations.

In any case, the currently available – largely phenomenological – evidence, taken together, suggests that the HF IP is generated and maintained during each anagen phase (and then disassembled again during the catagen and telogen phases) in order to sequester potentially deleterious, anagen- and/or melanogenesis-associated autoantigens from immune recognition by appropriately sensitized CD8+ T cells with cognate receptors, primarily via downregulation of MHC

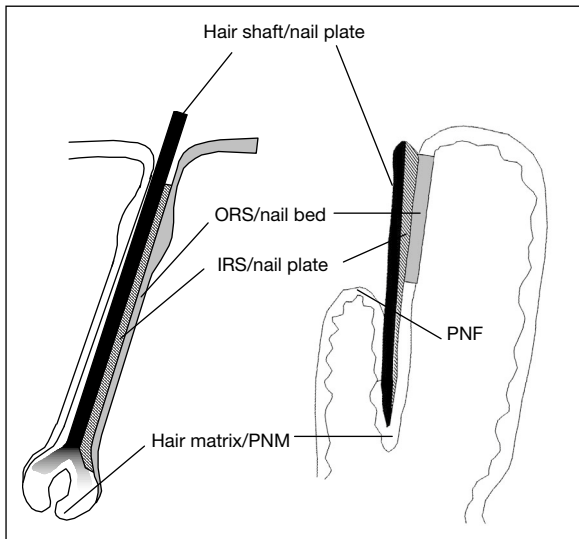


Fig. 7. The similarity of anatomical structure between HF and nail. Nail apparatus and HF share some anatomical features. Hair matrix, ORS and hair shaft are mirrored to PNM, nail bed and nail plate, respectively.

class I, and by the local production and secretion of potent immunosuppressants [76, 77].

Infantile Nail Matrix Is a Site of Relative IP

As indicated above, we have recently learned that the HF is not the only intracutaneous territory with an IP. The (stem cell-rich!) nail apparatus is often attacked by chronic inflammatory diseases (for example chronic eczema, lichen planus, psoriasis, AA, lupus erythematosus, scleroderma or bullous dermatoses), which may result in substantial, often irreversible changes to this functionally important skin appendage [78–81]. The nail apparatus is also constantly exposed to environmental damage, and thus requires a well-functioning immune response to combat infectious attack and tissue damage [79]. At the same time, not unlike the eye, a careful balance must be struck here between sufficient and undesired, autodestructive immune responses, if severe nail apparatus damage is to be avoided.

Given the substantial architectural similarities between HF and nail apparatus (nail and hair matrix are very similar; fig. 7), it was not surprising to

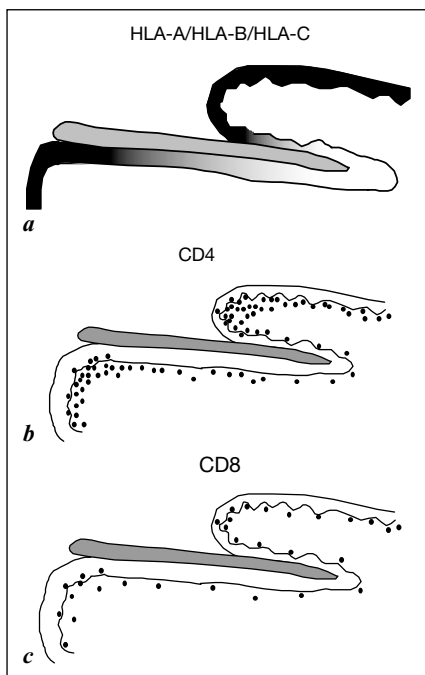


Fig. 8. MHC class I expression and distribution of CD4⁺ or CD8⁺ cells in the nail apparatus. There are some similarities in the expression of MHC class I and the distribution of CD4 and CD8 on nail apparatus compared to HF.

find that the, previously only ill-defined, nail apparatus immune system shows striking similarities with the HF immune system [12]. In particular, immunohistological analysis suggests that nail tissue also harbors a distinct area of IP. For example, HLA-A/HLA-B/HLA-C expression is prominently downregulated on both keratinocytes and melanocytes of the PNM, compared to other regions of the nail epithelium (fig. 8a). The PNM also shows moderate HLA-G IR and strong IR for locally generated immunosuppressants such as TGF- β 1, α -MSH and ACTH, as well as for macrophage migration-inhibitory factor as prominent inhibitor of NK cell activity. Around the PNM, there are only very few CD1a⁺, CD4⁺ or CD8⁺ cells, compared to nail fold and hyponychium (fig. 8b and c). CD1a⁺ cells in and around the PNM show reduced MHC class II and CD209 expression, indicating diminished antigen-presenting capacity [12].

Taken together, this suggests that the nail immune system strikingly differs from the skin immune system, but shows intriguing similarities to the HF immune system, including the establishment of an area of relative IP in the PNM. The functional advantages of establishing IP in the nail matrix are still

uncertain (for example, stringent containment of inflammation in a skin appendage that is notoriously under the attack of infectious agents and is prone to trauma-induced inflammatory tissue damage). Also, we do not know yet which key autoantigens are shared between nail apparatus and HFs, and whether the collapse of IP in the nail matrix may also underlie the frequent involvement of the nail in AA. However, it is already clear that our, as yet poor, understanding of the immunopathogenesis of nail growth disorders necessitates systemic dissection of the nail immune system and its IP.

The IP Collapse Model of AA Pathogenesis

While the pathogenesis of AA has still not been fully elucidated, recent consensus is building that AA reflects an organ-restricted, T cell-mediated autoimmune disease.

The most suggestive and important results in this respect were reported by Gilhar and Kalish [33] and Gilhar et al. [82], who demonstrated that AA lesions can be induced by the transfer of MHC class I-restricted CD8+ T cells alone, that anagen HF antigens are needed to stimulate T cells for effective triggering of AA lesions after cell transfer, and that these antigens can be substituted for by HF melanocyte antigens. The key histological feature of AA is a lymphocytic infiltrate around the lower HF, which may show a characteristic 'swarm of bees' pattern [83], but, in chronic AA, can also be much more discrete than widely assumed [84]. CD4+ T cells predominate in the infiltrate surrounding the HF, while T cells within the follicular epithelium are predominantly CD8+ [85].

In AA lesions, antigen-presenting cells, including Langerhans cells and macrophages, infiltrate the dystrophic HFs and often melanin deposition is observed around the dystrophic HFs [86]. Triggered by infectious foci, bacterial superantigens, psychoemotional stressors, skin microtrauma or other damage to the HF, and possibly aided by as yet ill-defined predisposing immunogenetic factors, a peri- and/or intrafollicular rise in AA IFN- γ secretion ectopically upregulates MHC class Ia expression in the proximal HF epithelium. Namely, this occurs in the normally MHC class I-negative hair matrix of anagen hair bulbs where active melanogenesis occurs (anagen III–VI) so that (notoriously immunogenic!) melanogenesis-associated antigens are massively generated, thus seriously endangering maintenance of the HF IP (fig. 9). Now follicular autoantigens can be ectopically presented in the normally MHC class I-negative epithelial hair bulb and are no longer sequestered.

Once the HF enters anagen and, at the latest, when its pigmentary unit engages in active melanogenesis (that is during anagen III/VI [87]), as yet obscure anagen- and/or melanogenesis-associated autoantigens are exposed to

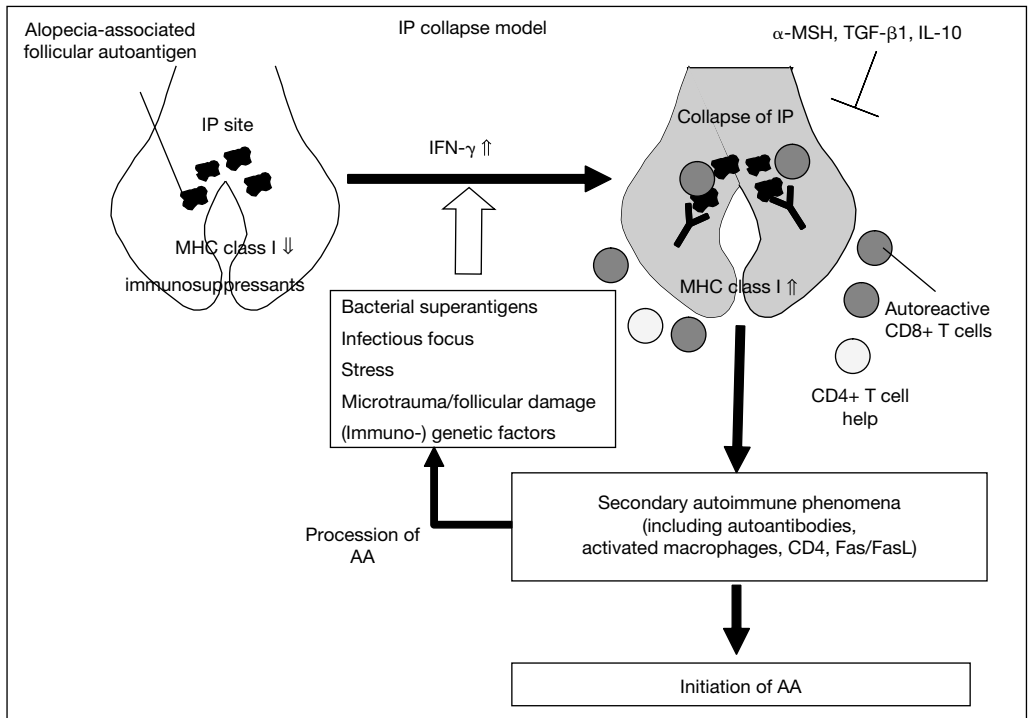


Fig. 9. The collapse model of HF IP: postulated immunopathogenesis of AA. Some endo-/exogenous factors induce IFN- γ production that upregulate MHC class I in hair bulb. Then, AA-associated follicular autoantigens are presented via MHC class I to autoreactive T cells that result in secondary autoimmune phenomena.

the skin immune system. In the event that a given individual has preexisting autoreactive CD8+ cells, which must receive appropriate costimulatory signals and help from CD4+ T cells (and possibly additional signals via CD4 as well), a cytotoxic T cell attack is launched on the hair matrix. This attack activates a vicious circle of secondary, follicle-damaging autoimmune phenomena, whose quality and magnitude largely determine the resulting degree of HF damage (dystrophy) and thus the actual clinical manifestation, progression and course of AA.

This basic hypothesis was later extended to account for a key role of locally generated immunosuppressants (such as α -MSH, TGF- β 1 and IGF-1 [60]) and NK cell-suppressing activities (for example MIF [68]) as 'guardians of HF IP', whose insufficient activity/function was thought to predispose

individuals towards AA development, while IP repair via these agents was suggested to underlie spontaneous AA remission and hair regrowth [11].

Upregulation of IFN- γ Is a Potent Trigger for the Induction of IP Collapse in the HF

IFN- γ -deficient mice are resistant to the development of AA [88]. We previously showed that, compared to IL-1 and TNF- α , IFN- γ offers the most potent cytokine stimulus for ectopic MHC class I expression in murine pelage HFs in vivo [63]. We recently succeeded in developing a standardized and highly reproducible in vitro assay that recreates the key feature of IP collapse postulated above in the human system: the ectopic upregulation of HLA-A/HLA-B/HLA-C expression in the matrix of normal human anagen scalp HFs. Using this new in vitro assay and very sensitive immunostaining techniques, confirmed by in situ hybridization and RT-PCR, we could show that IFN- γ is indeed a very potent stimulator of ectopic MHC class I expression in microdissected, organ-cultured human scalp HFs from healthy donors in anagen VI [89].

Low-dose IFN- γ can nicely be exploited experimentally to induce IP collapse of normal human anagen HFs in vitro, likely via an interferon regulatory factor-1-mediated mechanism [60]. Given that higher doses of IFN- γ also act as potent catagen inducer in human scalp HFs [89], this Th1 cytokine is a prime suspect as ‘match that lights the fire’ early during AA immunopathogenesis. The ability of IFN- γ to induce follicular MHC class I and II was used to test the hypothesis that AA results from loss of IP. C3H/HeJ female mice were injected intravenously with IFN- γ to induce follicular MHC. These injected mice demonstrated an increased rate of development of AA [32].

Restoration of HF IP

The new in vitro model allows screening for candidate agents that effectively downregulate IFN- γ -induced, ectopic MHC class I expression in human anagen HFs as the most important prerequisite for IP restoration. In fact, three immunomodulators known to be locally produced in the anagen hair bulb, α -MSH, TGF-1 and IGF-1 [49–52], are all capable of downregulating ectopic MHC class Ia expression, on both the protein and the mRNA level, when added to the culture medium after IFN- γ administration [89]. We are encouraged and intrigued by these findings because they provide a pragmatic and effective perspective on how IP restoration in AA can be achieved by administration of natural immunomodulators that the HF itself not only generates [49–52], but also

employs as key regulators of HF cycling during the anagen-catagen switch (that is TGF- β and IGF-1) [27, 47].

It is tempting to speculate that these same factors are also recruited by the anagen HF to maintain and restore its IP. In addition, the production/secretion of α -MSH, TGF- β , IGF-1, IL-10 and neutrophins (with the latter putatively being capable of deleting autoreactive CD8+ T cells via stimulation of the p75 neurotrophin receptor) may be upregulated whenever the HF becomes the target of immune injury and/or is threatened with a collapse of its IP. On the other hand, HF-derived α -MSH, TGF- β , IGF-1 and IL-10 – well-recognized natural immunosuppressants [7, 22, 90] – may efficiently dampen the secondary autoimmune phenomena that we envision as driving force behind the specific clinical characteristics and the progression of AA in any given patient [69].

Rather than targeting the secondary autoimmune phenomena associated with AA, as most of our currently employed treatment regimens continue to do, the primary goal of effective AA management must be to restore the HF's lost or compromised IP – both for preventing the progression of AA lesions and for inducing hair regrowth. IP restoration therapy does not require any prior knowledge of the relevant key autoantigens or the specific autoreactive T cells, and it can resort to the administration of well-known nonspecific immunomodulators that chiefly downregulate ectopic MHC class I expression in the anagen hair bulb.

The therapeutic use of powerful natural immunosuppressants such as α -MSH and TGF- β , along with the anagen-promoting and catagen-suppressing (as well as MHC class I-suppressing) cytokine IGF-1, all of which are locally generated in the HF itself and may be part of the follicle's own 'IP restoration machinery', promises to tilt the balance in favor of successful IP restoration. These agents also promise to carry with them minimal risks of toxicity, which may be further reduced by their topical application (for example via HF-targeted liposome preparations).

Perspectives

Systematic study of HF IP in human HF organ culture has already revealed promising candidate 'HI IP protectants' that, together with appropriate receptor agonists, offer themselves for the development of innovative management strategies for AA and other autoimmune diseases in which IP collapse plays an important role. In this context, it is particularly encouraging to note that a potent, clinically widely employed immunosuppressant, the immunophilin ligand FK 506 (tacrolimus), has already been documented to prevent and counteract IFN- γ -induced IP collapse in organ-cultured human anagen hair bulbs [60].

Thus, besides α -MSH, TGF- β 1 and IGF-1, we already hold a promising candidate downregulator of ectopic MHC class I expression in human scalp HFs in vivo in our hands, which deserves much more comprehensive clinical study in the context of AA management than it has received so far. Hopefully, this may pave the way for developing more effective immunotherapy strategies also for other autoimmune disorders where ectopic MHC class I expression and IP collapse play a pivotal role.

In terms of basic research on HF IP, we now need to learn which of the additional IP mechanisms recognized, for example in the context of ocular or fetomaternal IP, that have not yet been studied in the context of HF IP, are also relevant in the latter. Also, how many of the immune evasion strategies employed by viruses or malignant tumors [91–93] are also exploited by the HF to establish defined zones of relative IP? Furthermore, we need to know how the employment of these mechanisms differs between distinct HF subpopulations in different regions of the integument. Last but not least, it will be critical to elaborate how individuals with distinct immunogenetic backgrounds (including different genetic risk for the development of AA and other autoimmune diseases) differ in the constitutive feature of their HF IP and their (closely related) NK cell status.

Together, this will not only allow major advances in the therapy of the prototypic HF IP collapse disease in human skin, AA, but also provide invaluable new insights into the general characteristics, maintenance, collapse and restoration of IP – by systematically exploiting the most readily accessible and most abundantly available miniorgan of the mammalian body that displays IP.

References

- 1 Greene HSN: The use of the mouse eye in transplantation experiments. *Cancer Res* 1947;7: 491–501.
- 2 Andrew L, David M, Munn H: Immune privilege: a recurrent theme in immunoregulation? *Immunol Rev* 2006;213:5–11.
- 3 Billingham RE, Boswell T: Studies on the problem of corneal homografts. *Proc R Soc Lond B Biol Sci* 1953;141:392–406.
- 4 Head JR, Billingham RE: Immunologically privileged sites in transplantation immunology and oncology. *Perspect Biol Med* 1985;29:115–131.
- 5 Streilein JW: Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. *Curr Opin Immunol* 1993;5:428–432.
- 6 Brent L: *A History of Transplantation Immunology*. San Diego, Academic Press, 1997.
- 7 Janeway CA, Travers P, Walport M, Shlomchik MJ: *Autoimmunity and transplantation*; in Janeway CA (ed): *Immunobiology*. New York, Garland Publishing, 2001, pp 557–612.
- 8 Niederkorn JY: Immune privilege in the anterior chamber of the eye. *Crit Rev Immunol* 2002;22: 13–46.
- 9 Mellor AL, Munn DH: Immunology at the maternal-fetal interface: lessons for T cell tolerance and suppression. *Ann Rev Immunol* 2000;18:367–391.
- 10 Erlebacher A: Why isn't the fetus rejected? *Curr Opin Immunol* 2001;13:590–593.

- 11 Paus R, Nickoloff BJ, Ito T: A 'hairy' privilege. *Trends Immunol* 2005;26:32–40.
- 12 Ito T, Ito N, Saathoff M, Stampachiacchiere B, Bettermann A, Bulfone-Paus S, Takigawa M, Nickoloff BJ, Paus R: Immunology of the human nail apparatus: the nail matrix is a site of relative immune privilege. *J Invest Dermatol* 2005;125:1139–1148.
- 13 Bruno R, Sabater L, Sospedra M, Ferrer-Francesch X, Escudero D, Martinez-Caceres E, Pujol-Borrell R: Multiple sclerosis candidate autoantigens except myelin oligodendrocyt glycoprotein are transcribed in human thymus. *Eur J Immunol* 2002;32:2737–2747.
- 14 Filippini A, Riccioli A, Padula F, Lauretti P, D'Alessio A, De Cesaris P, Gandini L, Lenzi A, Ziparo E: Control and impairment of immune privilege in the testis and in semen. *Hum Reprod Update* 2001;7:444–449.
- 15 Lobo-Yeo A, Senaldi G, Portmann B, Mowat AP, Mieli-Vergani G, Vergani D: Class I and class II major histocompatibility complex antigen expression on hepatocytes: a study in children with liver disease. *Hepatology* 1990;12:224–232.
- 16 Franco A, Barnaba V, Natali P, Balsano C, Musca A, Balsano F: Expression of class I and class II major histocompatibility complex antigens on human hepatocytes. *Hepatology* 1988;8:449–454.
- 17 Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarselli B, Bencini E, Menicucci A, Baricordi OR: HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. *Eur J Immunol* 2002;32:311–315.
- 18 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;281:1191–1193.
- 19 Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P: Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 2003;4:1206–1212.
- 20 Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H: A critical role for murine complement regular Crry in fetomaternal tolerance. *Science* 2000;287:498–501.
- 21 Hobbs NK, Bondareva AA, Barnett S, Capecchi MR, Schmidt EE: Removing the vertebrate-specific TBP N terminus disrupts placental $\beta 2m$ -dependent interactions with the maternal immune system. *Cell* 2002;12:110:43–54.
- 22 Paul W: *Fundamental Immunology*. Philadelphia, Lippincott-Raven, 1999, p 49.
- 23 Tafuri A, Alferink J, Moller P, Hammerling GJ, Arnold B: T cell awareness of paternal alloantigens during pregnancy. *Science* 1995;270:630–633.
- 24 Meyer KV, Kloepper JE, Tiede S, Paus R: The human hair follicle bulge: immunohistological examination of potential stem cell-niche element, and further indications that it displays relative immune privilege. *J Invest Dermatol* 2007;127:A198.
- 25 Paus R, Pekar S: Biology of hair and nails; in Bologna JL, Jorizzo J, Rapini R (eds): *Dermatology*. London, Elsevier Science, 2003, vol 1, pp 1007–1032.
- 26 Paus R, Cotsarelis G: The biology of hair follicles. *N Engl J Med* 1999;341:491–498.
- 27 Cotsarelis G, Millar SE: Towards a molecular understanding of hair loss and its treatment. *Trends Mol Med* 2001;7:293–301.
- 28 Lang KS, Caroli CC, Muhm A, Wernet D, Moris A, Schitteck B, Knauss-Scherwitz E, Stevanovic S, Rammensee HG, Garbe C: HLA-A2 restricted, melanocyte-specific CD8⁺ T lymphocytes detected in vitiligo patients are related to disease activity and are predominantly directed against MelanA/MART1. *J Invest Dermatol* 2001;116:891–897.
- 29 Ogg GS, Rod Dunbar P, Romero P, Chen JL, Cerundolo V: High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. *J Exp Med* 1998;188:1203–1208.
- 30 Yagi H, Hashizume H, Horibe T, Yoshinari Y, Hata M, Ohshima A, Ito T, Takigawa M, Shibaki A, Shimizu H, Seo N: Induction of therapeutically relevant cytotoxic T lymphocytes in humans by percutaneous peptide immunization. *Cancer Res* 2006;66:10136–10144.
- 31 Barnhill RL, Llewellyn K: Benign melanocyte neoplasms; in Bologna JL, Jorizzo JL, Rapini RP (eds): *Dermatology*. London, Elsevier Science, 2003, pp 1757–1787.
- 32 Gilhar A, Kam Y, Assy B, Kalish RS: Alopecia areata induced in C3H/HeJ mice by interferon- γ : evidence for loss of immune privilege. *J Invest Dermatol* 2005;124:288–289.
- 33 Gilhar A, Kalish RS: Alopecia areata: a tissue specific autoimmune disease of the hair follicle. *Autoimmun Rev* 2006;5:64–69.

- 34 Billingham RE, Silvers WK: A biologist's reflections on dermatology. *J Invest Dermatol* 1971;57:227–240.
- 35 Billingham RE: The immunobiology of tissue transplantation. *Int Dent J* 1971;21:478–487.
- 36 Paus R, Stenn KS, Link RE: Telogen skin contains an inhibitor of hair growth. *Br J Dermatol* 1990;122:777–778.
- 37 Hofmann U, Tokura Y, Rückert R, Paus R: The anagen hair cycle induces systemic immunosuppression of contact hypersensitivity in mice. *Cell Immunol* 1998;184:65–73.
- 38 Tokura Y, Hofmann U, Müller-Röver S, Paus R, Wakita H, Yagi H, Seo N, Furukawa F, Takigawa M: Spontaneous hair follicle cycling may influence the development of murine contact photosensitivity by modulating keratinocyte cytokine production. *Cell Immunol* 1997;178:172–179.
- 39 Welker P, Foitzik K, Bulfone-Paus S, Henz BM, Paus R: Hair cycle-dependent changes in the gene expression and protein content of transforming factor $\beta 1$ and $v3$ in murine skin. *Arch Dermatol Res* 1997;289:554–557.
- 40 Slominski A, Botchkareva NV, Botchkarev VA, Chakraborty A, Luger T, Uenalan M, Paus R: Hair cycle-dependent production of ACTH in mouse skin. *Biochim Biophys Acta* 1998;1448:147–152.
- 41 Harrist TJ, Ruitter DJ, Mihm MC Jr, Bhan AK: Distribution of major histocompatibility antigens in normal skin. *Br J Dermatol* 1983;109:623–633.
- 42 Bröcker EB, Echternacht-Happle K, Hamm H, Happle R: Abnormal expression of class I and class II major histocompatibility antigen in alopecia areata. *J Invest Dermatol* 1987;88:564–568.
- 43 Westgate GE, Craggs RI, Gibson WT: Immune privilege and hair growth. *J Invest Dermatol* 1991;97:417–420.
- 44 Paus R, Eichmüller S, Hofmann U, Czarnetzki BM, Robinson P: Expression of classical and non-classical MHC class I antigens in murine hair follicles. *Br J Dermatol* 1994;131:177–183.
- 45 Christoph T, Müller-Röver S, Audring H, Tobin DJ, Hermes B, Cotsarelis G, Rückert R, Paus R: The human hair follicle immune system: cellular composition and immune privilege. *Br J Dermatol* 2000;142:862–873.
- 46 Paus R, Hofmann U, Eichmüller S, Czarnetzki BM: Distribution and changing density of $\gamma\delta$ T cells in murine skin during the induced hair cycle. *Br J Dermatol* 1994;130:281–289.
- 47 Stenn KS, Paus R: Control of hair follicle cycling. *Physiol Rev* 2001;81:449–494.
- 48 Foitzik K, Lindner G, Müller-Röver S, Maurer M, Botchkareva N, Botchkarev V, Handjiski B, Metz M, Hibino T, Soma T, Dotto GP, Paus R: Control of murine hair follicle regression (catagen) by TGF- $\beta 1$ in vivo. *FASEB J* 2000;14:752–760.
- 49 Slominski A, Wortsman J, Mazurkiewicz JE, Matsuoka L, Dietrich J, Lawrence K, Gorbani A, Paus R: Detection of proopiomelanocortin-derived antigens in normal and pathologic human skin. *J Lab Clin Med* 1993;122:658–666.
- 50 Slominski A, Wortsman J, Luger T, Paus R, Solomon S: Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. *Physiol Rev* 2000;80:979–1020.
- 51 Paus R, Botchkarev VA, Botchkareva NV, Mecklenburg L, Luger T, Slominski A: The skin POMC system (SPS): leads and lessons from the hair follicle. *Ann NY Acad Sci* 1999;885:350–363.
- 52 Botchkarev VA, Botchkareva NV, Slominski A, Roloff B, Luger T, Paus R: Developmentally regulated expression of α -MSH and MC-1 receptor in C57BL/6 mouse skin suggests functions beyond pigmentation. *Ann NY Acad Sci* 1999;885:433–439.
- 53 Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F, Straub RH, Paus R: Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. *FASEB J* 2005;19:1332–1334.
- 54 Foitzik K, Spexard T, Nakamura M, Halsner U, Paus R: Towards dissecting the pathogenesis of retinoid-induced hair loss: all-trans retinoic acid induces premature hair follicle regression (catagen) by upregulation of transforming growth factor- $\beta 2$ in the dermal papilla. *J Invest Dermatol* 2005;124:1119–1126.
- 55 Ohyama M, Terunuma A, Tock CL, Radomovich MF, Pise-Masison CA, Hopping SB, Brady JN, Udey MC, Vogel JC: Characterization and isolation of stem cell-enriched human hair follicle bulge cells. *J Clin Invest* 2006;116:249–260.
- 56 Morris JR, Liu Y, Marles L, Yang Z, Trempus C, Li S, Lin JS, Sawicki JA, Cotsarelis G: Capturing and profiling adult hair follicle stem cells. *Nat Biotech* 2004;22:411–417.

- 57 Gielen V, Schmitt D, Thivolet J: HLA class I antigen (heavy and light chain) expression by Langerhans cells and keratinocytes of the normal human epidermis: ultrastructural quantitation using immunogold labelling procedure. *Arch Dermatol Res* 1998;280:131–136.
- 58 Le Gal FA, Riteau B, Sedlik C, Khalil-Daher I, Menier C, Dausset J, Guillet JG, Carosella ED, Rouas-Freiss N: HLA-G-mediated inhibition on antigen-specific cytotoxic T lymphocytes. *Int Immunol* 1999;11:1351–1356.
- 59 Paus R, van der Veen C, Eichmüller S, Kopp T, Hagen E, Müller-Röver S, Hofmann U: Generation and cyclic remodeling of the hair follicle immune system in mice. *J Invest Dermatol* 1998;111:7–18.
- 60 Ito T, Ito N, Bettermann A, Tokura Y, Takigawa M, Paus R: Collapse and restoration of MHC class-I-dependent immune privilege: exploiting the human hair follicle as a model. *Am J Pathol* 2004;164:623–634.
- 61 Pamer E, Cresswell P: Mechanisms of MHC class I-restricted antigen processing. *Annu Rev Immunol* 1998;16:323–358.
- 62 Momburg F, Roelse J, Hammerling GJ, Neefjes JJ: Peptide size selection by the major histocompatibility complex-encoded peptide transporter. *J Exp Med* 1994;179:1613–1623.
- 63 Rückert R, Hofmann U, van der Veen C, Bulfone-Paus S, Paus R: MHC class I expression in murine skin: developmentally controlled and strikingly restricted intraepithelial expression during hair follicle morphogenesis and cycling, and response to cytokine treatment in vivo. *J Invest Dermatol* 1998;111:25–30.
- 64 Long EO: Regulation of immune responses through inhibitory receptors. *Annu Rev Immunol* 1999;17:875–904.
- 65 Vivier E, Tomasello E, Paul P: Lymphocyte activation via NKG2D: towards a new paradigm in immune recognition? *Curr Opin Immunol* 2002;14:306–311.
- 66 Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T: Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 1999;285:727–729.
- 67 Wu J, Song Y, Bakker AB, Bauer S, Spies T, Lanier LL, Phillips JH: An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* 1999;285:730–732.
- 68 Ito T, Saathoff M, Nickoloff BJ, Takigawa M, Paus R: Novel aspects of hair follicle immune privilege and their relevance to alopecia areata. *J Invest Dermatol* 2005;124:A103.
- 69 Slominski A, Paus R, Mazurkiewicz J: Proopiomelanocortin expression in the skin during induced hair growth in mice. *Experientia* 1992;48:50–54.
- 70 Cooper A, Robinson SJ, Pickard C, Jackson CL, Friedmann PS, Healy E: α -Melanocyte-stimulating hormone suppresses antigen-induced lymphocyte proliferation in humans independently of melanocortin 1 receptor gene status. *J Immunol* 2005;175:4806–4813.
- 71 Cerwenka A, Swain SL: GF- β 1: immunosuppressant and viability factor for T lymphocyte. *Microbes Infect* 1999;1:1291–1296.
- 72 Niederkorn JY: Mechanisms of immune privilege in the eye and hair follicle. *J Invest Dermatol Symp Proc* 2003;8:168–172.
- 73 Streilein JW: Ocular immune privilege: therapeutic opportunities from an experiment of nature. *Nature Rev Immunol* 2003;3:879–889.
- 74 Dawber R, Fenton DA: Infections and infestations; in Dawber R (ed): *Diseases of the Hair and Scalp*, ed 3 (rev). Oxford, Blackwell Science, 1997, pp 418–460.
- 75 Hermes B, Paus R: Scar forming alopecia: comments in classification, differential diagnosis and pathobiology. *Hautarzt* 1998;49:462–472.
- 76 Paus R, Christoph T, Müller-Röver S: Immunology of the hair follicle: a short journey into terra incognita. *J Invest Dermatol Symp Proc* 1999;4:226–234.
- 77 Paus R, Slominski A, Czarnetzki BM: Is alopecia areata an autoimmune-response against melanogenesis-related proteins, exposed by abnormal MHC class I expression in the anagen hair bulb? *Yale J Biol Med* 1994;66:541–554.
- 78 de Berker DAR, Baran R, Dawber RPR: The nail in dermatological diseases; in Baran R, Dawber RPR, de Berker DAR, Haneke E, Tosti A (eds): *Diseases of the Nails and their Management*. Oxford, Blackwell Science, 2001, pp 172–222.
- 79 Hanno R: Inflammatory reactions of the nail; in Farmer ER, Hood AF (eds): *Pathology of the Skin*. New York, McGraw-Hill, 2000, pp 870–875.

- 80 Tosti A, Baran R, Dawber RPR: The nail in systemic diseases and drug-induced changes; in Baran R, Dawber RPR, de Berker DAR, Haneke E, Tosti A (eds): *Diseases of the Nails and their Management*. Oxford, Blackwell Science, 2001, pp 223–229.
- 81 Tosti A, Piraccini BM: Biology of nails; in Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI (eds): *Fitzpatrick's Dermatology in General Medicine*. New York, McGraw-Hill, 2003, pp 159–163.
- 82 Gilhar A, Ullmann Y, Berkutzi T, Assy B, Kalish RS: Autoimmune hair loss (alopecia areata) transferred by T lymphocytes to human scalp explants on SCID mice. *J Clin Invest* 1998;101: 62–67.
- 83 Sperling LC, Lupton GP: Histopathology of non-scarring alopecia. *J Cutan Pathol* 1995;22: 97–114.
- 84 Whiting DA: Histopathologic features of alopecia areata: a new look. *Arch Dermatol* 2003;139: 1555–1559.
- 85 Todes-Taylor N, Turner R, Wood GS, Stratte PT, Morhenn VB: T cell subpopulations in alopecia areata. *J Am Acad Dermatol* 1984;11:216–223.
- 86 Hordingsky MK: Alopecia areata; in Olsen EA (ed): *Disorders of Hair Growth*. New York, McGraw-Hill, 2003, pp 239–274.
- 87 Slominski A, Paus R: Melanogenesis is coupled to murine anagen: toward new concepts for the role of melanocytes and the regulation of melanogenesis in hair growth. *J Invest Dermatol* 1993;101(suppl 1):90S–97S.
- 88 Freyschmidt-Paul P, McElwee KJ, Hoffmann R, Sundberg JP, Vitacolonna M, Kissling S, Zoller M: Interferon- γ -deficient mice are resistant to the development of alopecia areata. *Br J Dermatol* 2006;155:515–521.
- 89 Ito T, Ito N, Saathoff M, Bettermann A, Takigawa M, Paus R: Interferon- γ is a potent inducer of catagen-like changes in cultured human anagen hair follicles. *Br J Dermatol* 2005;152:623–631.
- 90 Luger TA, Scholzen T, Brzoska T, Becher E, Slominski A, Paus R: Cutaneous immunomodulation and coordination of skin stress responses by α -melanocyte-stimulating hormone. *Ann NY Acad Sci* 1998;840:381–394.
- 91 Vilches C, Parham P: KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Ann Rev Immunol* 2002;20:217–251.
- 92 Reddehase MJ: Antigens and immunoevasins: opponents in cytomegalievirus immune surveillance. *Nat Rev Immunol* 2002;2:831–844.
- 93 Pardoll D: Does the immune system see tumors as foreign or self? *Ann Rev Immunol* 2003;21: 807–839.

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Immunobiology of Acute Cytotoxic Drug Reactions

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Abstract

While relatively rare, the clinical presentation of toxic epidermal necrolysis (TENS) is often very dramatic and unfortunately it frequently results in the loss of life due to extensive epidermal necrosis and subsequent complications. The etiology of TENS is fairly well known and linked to ingestion of various medications. However, the pathophysiology and treatment of TENS is less well understood, but abnormal immunological mechanisms have been implicated by some investigators. The purpose of this chapter is to review the clinical features of TENS, followed by dermatopathological and immunopathological aspects of the skin lesions. A review of basic skin biology as regards regulation of keratinocytes' life and death is provided with emphasis on premature cell death. After a review of the immunopathogenic theories for TENS, a discussion of possible therapeutic interventions concludes the chapter. Clearly, many new insights are required at multiple levels of understanding to better manage and perhaps even prevent TENS.

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Toxic epidermal necrolysis syndrome (TENS), first described in 1956, represents a dramatic and often fatal clinical disease in which there is premature cell death of epidermal keratinocytes that generally follows the ingestion of a medication being given for a nonskin disease [1]. Drugs typically given to patients with seizures or infections such as phenytoin or sulfonamides are frequently associated with the onset of TENS. When patients experience this idiosyncratic drug reaction, their skin begins to slough and after several days following the onset of symptoms, the skin resembles that of individuals that have been scalded or suffered a superficial thermal injury [2]. Perhaps the most closely related disease that also features prominent epidermal keratinocyte cell death is acute cutaneous graft-versus-host disease seen in patients undergoing bone marrow transplantation.

While at first glance, it may not seem appropriate to include TENS in a series of chapters focusing on autoimmune diseases of the skin, it is possible to view the pathophysiology of this disease from an immunobiological perspective [3].

One important clue to suggest the involvement of the immune system and immunocytes in TENS patients comes from the recognition that the mortality for TENS patients is higher than that seen in burn patients, when the total body surface area of involvement is taken into consideration [3, 4]. This observation alone would suggest some other factors beyond just epidermal cell death need to be considered for TENS patients. Indeed, there are currently a wide variety of theories that have been proposed for the pathogenesis of TENS [4–12]. These theories range from a direct toxic effect of the ingested drug (or a metabolite) by which keratinocytes are directly induced to undergo cell death, to an allergic-type theory by which the drug binds to major histocompatibility complex on antigen-presenting cells and thereby activates autologous cytotoxic T cells which attack the keratinocytes, producing indirect cytopathic reactions in the epidermis. It is this latter viewpoint that most closely justifies inclusion of this disease in this series of articles. We will come back to this important point in a subsequent section where we review the various pathogenetic theories of TENS, but for the moment a focus on the histopathology of skin lesions, and the actual downstream pathological events in the epidermis will be presented in the next two sections. Prior to the next two sections, it is worth highlighting that the other disease process that features extensive epidermal keratinocyte cell death is acute cutaneous graft-versus-host disease which clearly has an immunological basis [13].

Basic Dermatopathological and Immunopathological Features of TENS

From a clinical perspective, there are several different diseases which must be considered in the differential diagnosis of TENS, including staphylococcal scalded skin syndrome (SSSS), erythema multiforme and Stevens-Johnson syndrome to name a few [14, 15]. To the noncognoscenti it should be emphasized that it is critically important to distinguish between TENS and SSSS, because both diseases can be fatal, but the therapeutic approaches are different. For example, a clinician would prescribe antibiotics to a patient with SSSS to target the bacteria producing the toxin that triggers epidermal sloughing. However, if the diagnosis is TENS, the clinician would consider taking the patient off an antibiotic. The actual histological distinction between SSSS and TENS is fairly easy when skin biopsies are examined. In SSSS, the histopathology is characterized by superficial sloughing of epidermal layers, whereas in TENS there is a panepidermal necrotic reaction [14]. Beyond the level of epidermal

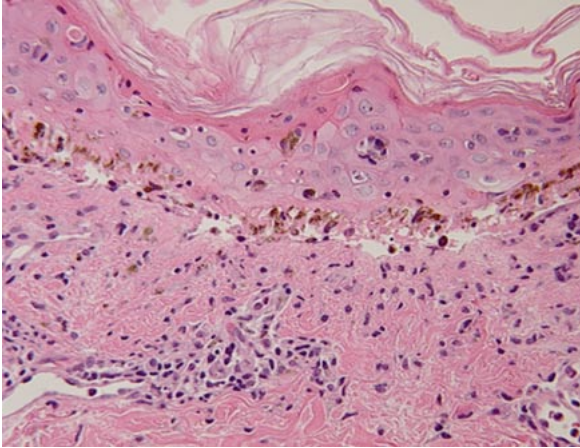


Fig. 1. High-power microscopic view of a hematoxylin and eosin-stained section of a typical TENS lesion reveals panepidermal keratinocyte cell death with loss of the basal cell layer and a relatively sparse superficial perivascular mononuclear cell infiltrate including lymphocytes, dendritic cells and macrophages.

keratinocytes undergoing necrosis, sloughing or apoptosis, another important light-microscopic feature for distinguishing between TENS and other skin disorders is the immunopathological features of the inflammatory cell infiltrate.

In TENS lesions, the traditional or textbook histological characteristics include a paucicellular inflammatory cell infiltrate by which relatively few mononuclear cells (such as T cells, macrophages and dendritic cells) are accompanied by extensive keratinocyte destruction via apoptosis with dermal-epidermal separation and potential blister formation and full thickness epidermal sloughing [16]. In figure 1, a representative light-microscopic view of a TENS lesion is portrayed. Note the extensive epidermal necrosis with complete destruction of the basal cell layer producing a slight cleft between the epidermal and dermal compartments. There is a slight superficial peri-vascular lymphocytic inflammatory infiltrate accompanied by interstitial infiltrate of mononuclear cells. By contrast to TENS skin lesions, the microscopic pathologies of erythema multiforme and Stevens-Johnson syndrome lesions typically display less epidermal keratinocyte cytotoxicity and a more prominent and diffuse dermal mononuclear cell infiltrate that includes obscuring the dermal-epidermal interface and extension of the T cells into the epidermal compartment. As many cases of erythema multiforme are associated with herpes virus infection, the extensive mononuclear cell infiltrate can be understood in the context of a delayed-type hypersensitivity reaction by which viral antigens are expressed on the epidermal keratinocyte [17, 18].

Regarding the quantity and quality of the cellular inflammatory infiltrate in TENS, less attention has been devoted to those cases in which a biopsy does not reveal a paucicellular infiltrate. In a large series of cases comparing TENS to erythema multiforme and Stevens-Johnson syndrome, skin lesions of TENS with intermediate or extensive inflammation were found in 5 of 23 patients for a relative value of 22% [19]. In our recently published series of carefully documented TENS patients, we categorized skin lesions by the extent of inflammation into either paucicellular or sparse, moderate, or extreme [20]. The mean cell counts (± 1 SD) for each category were as follows: sparse = 161 cells/high power field (hpf); moderate = 273 cells/hpf; extensive = 392 cells/hpf. Of the 37 patients with TENS analyzed, there were 15 patients with sparse inflammatory infiltrates, but also 15 patients with moderate inflammation and 7 patients with extensive inflammation.

Perhaps of greater importance in our study was the observation that there was a significant correlation between the patient outcome and the extent of inflammation. Thus, even though all patients had at least 30% body surface area involvement, individuals with more inflammation had a higher mortality rate. This outcome was unexpected because as stated earlier, patients with erythema multiforme and Stevens-Johnson syndrome have prominently inflamed skin lesions but very low mortality rates compared with these other skin disorders (some of which may also be associated with drug ingestion). Such an unexpected clinical-pathological finding led us to perform additional immunohistochemical staining and other clinical correlations with statistical analysis.

By immunostaining, previous investigators characterized the early cellular immune infiltrate of TENS lesions [21, 22]. Examining either tissue samples or blister fluid of TENS patients, the primary T cell subset is characterized as a CD8+ T cell that is accompanied by T cells also expressing natural killer antigens [23, 24]. Since there are only rare CD8+ T cells in lesional epidermis [25], there may be a role for secreted factors or soluble mediators that trigger premature cell death in epidermal keratinocytes. Besides CD8+ T cells, we also identified increased numbers of CD4+ T cells, as well as cytotoxic T cells expressing TIA-1 (a marker for cytotoxicity [26]) accompanied by factor XIIIa-positive dermal dendrocytes [20].

Premature Epidermal Keratinocyte Death in TENS Patients

As mentioned above, the preeminent clinical and histological change in the skin of patients with TENS is acute cytotoxic dermatitis. The concept of premature cell death when considering the biology of the epidermis requires

highlighting of this topic for several reasons [27]. First, one must recognize that the primary function of the skin is to serve as an effective barrier at the interface between the external environment and internal organ physiology. Such tissue homeostasis requires a barrier to be created and maintained and in the case of the epidermis, this barrier is subserved by the stratum corneum [28]. The stratum corneum is the most superficial layer of the epidermis and is composed of essentially dead cells or corneocytes that are formed when the granular cell layers representing the most terminally differentiated cells undergo cell death [29]. Thus, when keratinocytes leave the proliferative compartment along the basal layer adjacent to the underlying dermis, they begin to differentiate. This differentiation process culminates in the granular cell layer, and within an additional layer these terminally differentiated keratinocytes then undergo a complex and poorly understood death process by which all of the nuclear DNA and RNA is dissolved, accompanied by disappearance of all cytoplasmic organelles such as endoplasmic reticulum, ribosomes and mitochondria. In addition to these events, there is also cross-linking of the plasma membrane constituents by transglutaminase, forming a shroud-like structure that is highly rigid and impermeable to significant water fluxes or invasion by infectious agents [30].

Second, some investigators have likened the death process occurring at the uppermost layers of the epidermis to apoptosis or programmed cell death [31]. However, it is very unclear exactly what type of process is actually responsible for the death of corneocytes. Because of this uncertainty we have used the term 'planned cell death' to emphasize the notion that under physiological conditions, keratinocytes must initially differentiate before dying and that pathological processes basically involve the premature death of cells before they have differentiated [27].

Returning to TENS, the hallmark pathological feature is extensive premature cell death of keratinocytes throughout all cell layers of the epidermis [32]. This particular type of premature cell death does appear to be secondary to activation of caspases that are most closely considered as apoptosis [33]. One of the key questions to be delineated for TENS is exactly what type of apoptotic machinery is activated within the epidermis to generate such extensive keratinocyte destruction with loss of the barrier function, as well as dermal scar formation. There are basically 2 types of cell death pathways, the first known as the extrinsic pathway involving activation of death receptors as the initiating event, and the second known as the intrinsic pathway that features initiating events within the mitochondria. Of course the most important and unresolved aspect of this disease is how ingesting a medication such as dilantin, an antibiotic or some other drug given orally does culminate in such abrupt and often extensive epidermal keratinocyte apoptosis.

Pathogenic Theories Underlying Premature Keratinocyte Death in TENS Patients

As mentioned earlier, there are a wide variety of theories proposed for the pathogenesis of TENS. These theories include involvement of Fas:Fas ligand, cytotoxicity mediated by perforin and granzyme B, nitric oxide synthase, as well as cytokines such as IFN- γ and TNF- α . One of the leading pathophysiological theories for TENS involves the engagement of the cell death receptor pathway leading to premature apoptosis of epidermal keratinocytes. The basis for this proposal was the initial documentation of aberrant expression of a soluble form of Fas ligand (FasL) by the epidermal keratinocytes secondary to drug ingestion [34]. This FasL then binds to its death receptor, Fas antigen (CD95), activating intracellular signaling pathways involving Fas-associated protein with death domain and ultimately various proapoptotic caspases. Exactly what component of the ingested drug would lead to keratinocyte upregulation of CD95L is not clear. However, it has also been suggested that one of the mechanisms of action for a therapy that has been found to have some efficacy in some TENS patients is the administration of intravenous γ -globulin (such as intravenous immunoglobulin, IVIG) [8]. In this scenario the antibodies within IVIG would be capable of binding to a neutralizing soluble FasL and thus prevent the keratinocytes from committing suicide via the death receptor pathway [35]. Further elaboration on IVIG and other therapies for TENS patients is presented in the next section.

While the initial link between FasL and Fas provided new insights into the immunopathogenesis of TENS, several subsequent investigators have not consistently demonstrated similar findings. For example, one study did not identify FasL on lesional keratinocytes [5], or only identified FasL in the cytoplasm [36]. Another unresolved issue is whether FasL, if even present on lesional keratinocytes, could effectively engage the Fas receptor on adjacent keratinocytes to trigger apoptosis. While keratinocytes may overexpress Fas, they are significantly less susceptible to killing compared to the Jurkat T cell line used by Viard-Leveugle et al. [36] and others [37–38]. Besides these perplexing aspects of the Fas:FasL hypothesis for TENS, there is the issue related to the actual cellular source of production for soluble FasL. Even though several groups have detected soluble FasL in the circulation of TENS patients, at least one group has suggested it is derived from peripheral blood mononuclear cells, rather than epidermal keratinocytes [5].

Another theory implicates more of an allergic-type reaction to the ingested drug. The key concept for this immunological theory includes complementary observations made by several investigative teams. The first set of observations involved documenting the presence of cytotoxic-type T cells within the blister

fluid of TENS lesions [39, 40]. The lesional fluid was also characterized and found to contain high levels of TNF and IFN- γ [38]. While cytokines such as TNF and IFN- γ have only been associated with cytostatic but not overt cytotoxic reactions using human keratinocyte monolayer cultures, it is possible that different responses would be seen if three-dimensional reconstructed epidermal/dermal equivalents were used. It is also possible that there may be cross-talk between cytokines such as IFN- γ and TNF- α with soluble FasL [41]. Finally, an additional consideration for the pathophysiology of TENS is related to nitric oxide synthase by which increased nitric oxide would be responsible for the death of epidermal keratinocytes [42]. Given the uncertainty for the pathophysiology of TENS, it should not be surprising that currently used therapies are of limited efficacy, and the mechanism of action for several of these therapeutic modalities remains to be defined. In the next session, a brief review of this topic will be presented.

Therapeutic Intervention in TENS Patients

Before delving into specific therapeutic approaches to TENS, it is important to emphasize the relatively high mortality rate, the methods for evaluating TENS patients and the criteria that are useful for predicting mortality. First, it should be noted that most clinical centers admitting patients with TENS generally refer these individuals to intensive care units and burn units because they rapidly deteriorate and require wound care, fluid resuscitation and treatment of multiple organ failure and infectious complications [43].

Second, the mortality rates and morbidity figures for TENS patients should consider not only short-term, but also long-term outcomes [44]. Overall, the mortality range for TENS patients is 25–35% [45]. As regards predicting mortality, a validated predictor is referred to as the score for evaluation of toxic epidermal necrolysis (SCORTEN), which takes into consideration a variety of parameters [46]. These parameters include patient's age, presence of malignancy, heart rate, body surface area involved, serum urea levels, glucose and serum bicarbonate levels. When we compared the current gold standard for predicting mortality (SCORTEN) with the histological assessment based on the intensity of inflammation (as mentioned in a previous section), a similar statistical result was obtained for TENS patients with greater than 30% total body surface area involvement [20]. Whether this 'pathology score' should be added for SCORTEN patients remains to be determined [3].

Having reviewed the assessment criteria for evaluating TENS patients from a clinical perspective, the next topic to be covered is the efficacy of recently studied agents including IVIG, as well as cyclosporin A (CsA).

One of the most widely studied (and controversial) therapeutic approaches for TENS patients is the use of IVIG. The IVIG preparations used in these studies are derived from various sources and consist of a pool of immunoglobulins derived from healthy donors. As there is no defined agent or drug within this pooled biological material, there can be considerable variation in the actual concentration of any one type of antibody. In theory, IVIG is supposed to work by the binding of FasL via antibodies contained within this protective material. The use of IVIG was bolstered by the positive findings reported by Viard-Leveugle et al. [36]. During the past ten years, several reports have found widely divergent responses including both positive and negative clinical results [47–51]. At our own institution, it was found that IVIG was ineffective, and hence is no longer administered to TENS patients [52].

Another therapeutic approach involves the use of CsA. The rationale for using CsA is that it can inhibit cytotoxic T cell subsets such as CD8+ T cells. Even though it is not known why ingestion of a medication triggers cytotoxic T cell activity, we and others have detected T cells with cytotoxic markers and activity with TENS lesions, and hence it makes sense to try and use CsA. On the other hand, using a potent immunosuppressive agent in patients that have significant epidermal barrier defects and other systemic metabolic abnormalities may exacerbate the patients' susceptibility to infection. Not surprisingly, only a few clinical trials have been conducted. There was a trend to suggest a possible positive effect of CsA in TENS patients [53, 54].

Since abnormal cytokine networks are proposed for TENS, it will be interesting to determine if biological agents targeting TNF will be effective, or whether combining anti-TNF agents with either CsA and/or IVIG will demonstrate any efficacy in patients with TENS.

Targeting TNF may be worthy of therapeutic consideration as administration of anti-TNF antibody improved TENS [55], and thalidomide, pentoxifylline, N-acetylcysteine (agents which alter TNF levels) also have been used for TENS [56–58]. Whether the use of corticosteroids should be continued remains to be determined [59]. Basic wound care and supportive nutrition and infectious disease control are key components of care; and new types of skin substitutes are currently being developed and evaluated for TENS patients [60].

Conclusion

TENS is a complex disease that is one of the most dramatic examples of enhanced premature epidermal keratinocyte cell death in dermatology. Just about every aspect of this disease (beyond its link to ingestion of medications)

ranging from its immunopathology, pathophysiology and treatment remains enigmatic. Hopefully, as investigative skin biologists team up with clinicians involved in the care of TENS patients, new insights will emerge regarding its pathogenesis and treatment. Of course, it will also be worthwhile and relevant to follow developments involving acute cutaneous graft-versus-host disease for additional clues regarding the immunopathogenesis and treatment of TENS patients.

Acknowledgements

The author thanks Ms. Lori Kmet for manuscript preparation. This article was supported by NIH grants AR47314 and CA39542.

References

- 1 Lyell A: Toxic epidermal necrolysis: an eruption resembling scalding of the skin. *Br J Dermatol* 1956;68:355–361.
- 2 Roujeau JC: The spectrum of Stevens-Johnson syndrome and toxic epidermal necrolysis: a clinical classification. *J Invest Dermatol* 1994;102:28S–30S.
- 3 Ousmane F, Wechsler J, Roujeau JC: Cell-mediated immunologic mechanism and severity of TEN. *Arch Dermatol* 2005;141:775–776.
- 4 Monafo WW: Initial management of burns. *N Engl J Med* 1996;335:1581–1586.
- 5 Abe R, Shimizu T, Shibaki A, Nakamura H, Watanabe H, Shimizu H: Toxic epidermal necrolysis and Stevens-Johnson syndrome are induced by soluble Fas ligand. *Am J Pathol* 2003;162:1515–1520.
- 6 Caproni M, Torchia D, Schincaglia E, Volpi W, Frezzolini A, Schena D, Marzano A, Quaglini P, De Simone C, Parodi A, Barletta E, Fabbri P: Expression of cytokines and chemokine receptors in the cutaneous lesions of erythema multiforme and Stevens-Johnson syndrome/toxic epidermal necrolysis. *Br J Dermatol* 2006;155:722–728.
- 7 Inachi S, Mizutani H, Shimizu M: Epidermal apoptotic cell death in erythema multiforme and Stevens-Johnson syndrome: contribution of perforin-positive cell infiltration. *Arch Dermatol* 1997;133:845–849.
- 8 Khalili B, Bahna SL: Pathogenesis and recent therapeutic trends in Stevens-Johnson syndrome and toxic epidermal necrolysis. *Ann Allergy Asthma Immunol* 2006;97:272–280.
- 9 Paquet P, Pierard GE: Glutathione-S-transferase pi expression in toxic epidermal necrolysis: a marker of putative oxidative stress in keratinocytes. *Skin Pharmacol Physiol* 2006;20:66–70.
- 10 Roujeau JC, Gelard K, Bensussan A: Epidermal necrolysis: mechanisms of keratinocyte apoptosis. *Med Sci (Paris)* 2006;22:188–191.
- 11 Teraki Y, Hitomi K, Sato Y, Hamamatsu Y, Izaki S: Leflunomide-induced toxic epidermal necrolysis. *Int J Dermatol* 2006;45:1370–1371.
- 12 Caproni M, Torchia D, Schincaglia E, Volpi W, Frezzolini A, Schena D, Marzano A, Quaglini P, De Simone C, Parodi A, Barletta E, Fabbri P: The CD40/CD40 ligand system is expressed in the cutaneous lesions of erythema multiforme and Stevens-Johnson syndrome/toxic epidermal necrolysis spectrum. *Br J Dermatol* 2006;154:319–324.
- 13 Hofmeister CC, Quinn A, Cooke KR, Stiff P, Nickoloff B, Ferrara JL: Graft-versus-host disease of the skin: life and death on the epidermal edge. *Biol Blood Marrow Transplant* 2004;10:366–372.
- 14 Coleman JC, Dobson NR: Diagnostic dilemma: extremely low birth weight baby with staphylococcal scalded-skin syndrome or toxic epidermal necrolysis. *J Perinatol* 2006;26:714–716.

- 15 Stanley JR, Amagai M: Pemphigus, bullous impetigo, and the staphylococcal scalded-skin syndrome. *N Engl J Med* 2006;355:1800–1810.
- 16 Paquet P, Pierard GE, Quatresooz P: Novel treatments for drug-induced toxic epidermal necrolysis (Lyell's syndrome). *Int Arch Allergy Immunol* 2005;136:205–216.
- 17 Aurelian L, Kokuba H, Burnett JW: Understanding the pathogenesis of HSV-associated erythema multiforme. *Dermatology* 1998;197:219–222.
- 18 Aurelian L, Ono F, Burnett J: Herpes simplex virus (HSV)-associated erythema multiforme (HAEM): a viral disease with an autoimmune component. *Dermatol Online J* 2003;9:1.
- 19 Rzany B, Hering O, Mockenhaupt M, Schroder W, Goerttler E, Ring J, Schopf E: Histopathological and epidemiological characteristics of patients with erythema exudativum multiforme major, Stevens-Johnson syndrome and toxic epidermal necrolysis. *Br J Dermatol* 1996;135:6–11.
- 20 Quinn AM, Brown K, Bonish BK, Curry J, Gordon KB, Sinacore J, Gamelli R, Nickoloff BJ: Uncovering histologic criteria with prognostic significance in toxic epidermal necrolysis. *Arch Dermatol* 2005;141:683–687.
- 21 Roujeau JC, Moritz S, Guillaume JC, Bombal C, Revuz J, Weil B, Touraine R: Lymphopenia and abnormal balance of T-lymphocyte subpopulations in toxic epidermal necrolysis. *Arch Dermatol Res* 1985;277:24–27.
- 22 Merot Y, Gravallesse E, Guillen FJ, Murphy GF: Lymphocyte subsets and Langerhans' cells in toxic epidermal necrolysis: report of a case. *Arch Dermatol* 1986;122:455–458.
- 23 Le Cleach L, Delaire S, Boumsell L, Bagot M, Bourgault-Villada I, Bensussan A, Roujeau JC: Blister fluid T lymphocytes during toxic epidermal necrolysis are functional cytotoxic cells which express human natural killer (NK) inhibitory receptors. *Clin Exp Immunol* 2000;119:225–230.
- 24 Correia O, Delgado L, Ramos JP, Resende C, Torrinha JA: Cutaneous T-cell recruitment in toxic epidermal necrolysis: further evidence of CD8+ lymphocyte involvement. *Arch Dermatol* 1993;129:466–468.
- 25 Miyauchi H, Hosokawa H, Akaeda T, Iba H, Asada Y: T-cell subsets in drug-induced toxic epidermal necrolysis: possible pathogenic mechanism induced by CD8-positive T cells. *Arch Dermatol* 1991;127:851–855.
- 26 Austin LM, Coven TR, Bhardwaj N, Steinman R, Krueger JG: Intraepidermal lymphocytes in psoriatic lesions are activated GMP-17(TIA-1)+CD8+CD3+ CTLs as determined by phenotypic analysis. *J Cutan Pathol* 1998;25:79–88.
- 27 Qin JZ, Chaturvedi V, Bonish B, Nickoloff BJ: Avoiding premature apoptosis of normal epidermal cells. *Nat Med* 2001;7:385–386.
- 28 Nickoloff BJ, Qin JZ, Chaturvedi V, Bacon P, Panella J, Denning MF: Life and death signaling pathways contributing to skin cancer. *J Invest Dermatol Symp Proc* 2002;7:27–35.
- 29 Lippens S, Denecker G, Ovaere P, Vandenabeele P, Declercq W: Death penalty for keratinocytes: apoptosis versus cornification. *Cell Death Differ* 2005;12(suppl 2):1497–1508.
- 30 Kalinin AE, Kajava AV, Steinert PM: Epithelial barrier function: assembly and structural features of the cornified cell envelope. *Bioessays* 2002;24:789–800.
- 31 Lamkanfi M, Festjens N, Declercq W, Berghe TV, Vandenabeele P: Caspases in cell survival, proliferation and differentiation. *Cell Death Differ* 2007;14:44–55.
- 32 Paquet P, Pierard GE: Toxic epidermal necrolysis: revisiting the tentative link between early apoptosis and late necrosis (review). *Int J Mol Med* 2007;19:3–10.
- 33 Paul C, Wolkenstein P, Adle H, Wechsler J, Garchon HJ, Revuz J, Roujeau JC: Apoptosis as a mechanism of keratinocyte death in toxic epidermal necrolysis. *Br J Dermatol* 1996;134:710–714.
- 34 Viard I, Wehrli P, Bullani R, Schneider P, Holler N, Salomon D, Hunziker T, Saurat JH, Tschopp J, French LE: Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science* 1998;282:490–493.
- 35 Mittmann N, Chan B, Knowles S, Cosentino L, Shear N: Intravenous immunoglobulin use in patients with toxic epidermal necrolysis and Stevens-Johnson syndrome. *Am J Clin Dermatol* 2006;7:359–368.
- 36 Viard-Leveugle I, Bullani RR, Meda P, Micheau O, Limat A, Saurat JH, Tschopp J, French LE: Intracellular localization of keratinocyte Fas ligand explains lack of cytolytic activity under physiological conditions. *J Biol Chem* 2003;278:16183–16188.

- 37 Paquet P, Pierard GE: Keratinocyte injury in drug-induced toxic epidermal necrolysis: simultaneous but distinct topographic expression of CD95R and calprotectin. *Int J Mol Med* 2002;10:145–147.
- 38 Nassif A, Moslehi H, Le Gouvello S, Bagot M, Lyonnet L, Michel L, Bousmell L, Bensussan A, Roujeau JC: Evaluation of the potential role of cytokines in toxic epidermal necrolysis. *J Invest Dermatol* 2004;123:850–855.
- 39 Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ: Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. *J Immunol* 1995;155:462–472.
- 40 Zanni MP, von Greysen S, Schnyder B, Brander KA, Frutig K, Hari Y, Valitutti S, Pichler WJ: HLA-restricted, processing- and metabolism-independent pathway of drug recognition by human alpha beta T lymphocytes. *J Clin Invest* 1998;102:1591–1598.
- 41 Schnyder B, Frutig K, Mauri-Hellweg D, Limat A, Yawalkar N, Pichler WJ: T-cell-mediated cytotoxicity against keratinocytes in sulfamethoxazol-induced skin reaction. *Clin Exp Allergy* 1998;28:1412–1417.
- 42 Lerner LH, Qureshi AA, Reddy BV, Lerner EA: Nitric oxide synthase in toxic epidermal necrolysis and Stevens-Johnson syndrome. *J Invest Dermatol* 2000;114:196–199.
- 43 Neff P, Meuli-Simmen C, Kempf W, Gaspert T, Meyer VE, Kunzi W: Lyell syndrome revisited: analysis of 18 cases of severe bullous skin disease in a burns unit. *Br J Plast Surg* 2005;58:73–80.
- 44 Ducic I, Shalom A, Rising W, Nagamoto K, Munster AM: Outcome of patients with toxic epidermal necrolysis syndrome revisited. *Plast Reconstr Surg* 2002;110:768–773.
- 45 Palmieri TL, Greenhalgh DG, Saffle JR, Spence RJ, Peck MD, Jeng JC, Mazingo DW, Yowler CJ, Sheridan RL, Ahrenholz DH, Caruso DM, Foster KN, Kagan RJ, Voigt DW, Purdue GF, Hunt JL, Wolf S, Molitor F: A multicenter review of toxic epidermal necrolysis treated in US burn centers at the end of the twentieth century. *J Burn Care Rehabil* 2002;23:87–96.
- 46 Bastuji-Garin S, Fouchard N, Bertocchi M, Roujeau JC, Revuz J, Wolkenstein P: SCORTEN: a severity-of-illness score for toxic epidermal necrolysis. *J Invest Dermatol* 2000;115:149–153.
- 47 Stella M, Cassano P, Bollero D, Clemente A, Giorio G: Toxic epidermal necrolysis treated with intravenous high-dose immunoglobulins: our experience. *Dermatology* 2001;203:45–49.
- 48 Prins C, Kerdell FA, Padilla RS, Hunziker T, Chimenti S, Viard I, Mauri DN, Flynn K, Trent J, Margolis DJ, Saurat JH, French LE: Treatment of toxic epidermal necrolysis with high-dose intravenous immunoglobulins: multicenter retrospective analysis of 48 consecutive cases. *Arch Dermatol* 2003;139:26–32.
- 49 Campione E, Marulli GC, Carrozzo AM, Chimenti MS, Costanzo A, Bianchi L: High-dose intravenous immunoglobulin for severe drug reactions: efficacy in toxic epidermal necrolysis. *Acta Derm Venereol* 2003;83:430–432.
- 50 Bachot N, Revuz J, Roujeau JC: Intravenous immunoglobulin treatment for Stevens-Johnson syndrome and toxic epidermal necrolysis: a prospective noncomparative study showing no benefit on mortality or progression. *Arch Dermatol* 2003;139:33–36.
- 51 Trent JT, Kirsner RS, Romanelli P, Kerdell FA: Analysis of intravenous immunoglobulin for the treatment of toxic epidermal necrolysis using SCORTEN: the University of Miami experience. *Arch Dermatol* 2003;139:39–43.
- 52 Brown KM, Silver GM, Halerz M, Walaszek P, Sandroni A, Gamelli RL: Toxic epidermal necrolysis: does immunoglobulin make a difference? *J Burn Care Rehabil* 2004;25:81–88.
- 53 Hewitt J, Ormerod AD: Toxic epidermal necrolysis treated with cyclosporin. *Clin Exp Dermatol* 1992;17:264–265.
- 54 Sullivan JR, Watson A: Lamotrigine-induced toxic epidermal necrolysis treated with intravenous cyclosporin: a discussion of pathogenesis and immunosuppressive management. *Australas J Dermatol* 1996;37:208–212.
- 55 Fischer M, Fiedler E, Marsch WC, Wohlrab J: Antitumour necrosis factor-alpha antibodies (infliximab) in the treatment of a patient with toxic epidermal necrolysis. *Br J Dermatol* 2002;146:707–709.
- 56 Namazi MR: Increased mortality in toxic epidermal necrolysis with thalidomide: corroborating or exonerating the pathogenetic role of TNF- α ? *Br J Dermatol* 2006;155:842–843.

- 57 Redondo P, Ruiz De Erenchum F, Iglesias ME: Toxic epidermal necrolysis. Treatment with pentoxifylline. *Br J Dermatol* 1994;130:688–689.
- 58 Velez A, Moreno JC: Toxic epidermal necrolysis treated with N-acetylcysteine. *J Am Acad Dermatol* 2002;46:469–470.
- 59 Schneck J, Fagot J-P, Sekula P, Math D, Sassolas B, Roujeau JC, Mockenhaupt M: Effects of treatments on the mortality of Stevens-Johnson syndrome and toxic epidermal necrolysis: A retrospective study on patients included in the prospective EuroSCAR study. *J Am Acad Dermatol* 2007, in press.
- 60 Boorboor P, Vogt PM, Bechara FG, Alkandari Q, Aust M, Gohritz A, Spies M: Toxic epidermal necrolysis: Use of Biobrane for skin coverage reduces pain, improves mobilisation and decreases infection in elderly patients. *Burns* 2007, in press.

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Psoriasis

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Abstract

Psoriasis is one of the most common chronic inflammatory disorders with a strong genetic background. Recent progress in the understanding of both the immunological as well as the genetic basis has provided an unprecedented opportunity to move scientific insights from the bench to bedside. Based on insights from laboratory research, targeted immunotherapies are now available for the benefit of patients suffering from psoriasis. The success of these therapies has validated insights into disease pathogenesis and also provides the opportunity to increase our understanding about the pathways underpinning autoimmune-type inflammation in the skin.

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Psoriasis is a chronic inflammatory skin disease based on polygenic predisposition [1]. Its main clinical subtype, plaque-type psoriasis, is defined by recurring erythematous, scaly plaques in often symmetric configuration. It was only in the 19th century that Willan (1809) gave an accurate description of psoriasis and Hebra (1841) defined it as a clinical entity distinct from leprosy [2]. While progress in the dissection of the molecular events leading to psoriasis already has a major impact on treatment, consequences for disease classification are much less advanced. Nearly every novel immunological cell or mediator has been (re-)discovered in fully established psoriasis lesions. However, these purely observational data are rarely put into a functional perspective, which is only possible in the context of relevant disease models and/or interventional proof or principle studies in patients. Thus, important issues such as disease-relevant pathways and therapeutic targets are only beginning to be understood in their full complexity. Major insights related to these topics derive

from recent breakthroughs in whole genome assessments of genetic risk variants, the psoriasis-specific transcriptome and proteome, as well as the development of novel disease-relevant humanized animal models.

Immunogenetics

Psoriasis is an inflammatory disorder based on the combined influence of predisposing gene variants, genetic modifiers and environmental factors. The strong influence of genes in psoriasis is supported by a three times higher concordance rate in monozygotic versus dizygotic twins [3]. Genome scans have identified at least 19 genetic susceptibility loci, but replication of single loci has been only provided for a few of those. Extensive replication has been provided for a genetic risk region in psoriasis patients confined to an approximately 160-kb stretch of DNA on the short arm of chromosome 6 (termed PSORS1). This region includes genes such as HLA-Cw6 as potential immunological candidate gene and corneodesmosin as a potential epidermal structure-related candidate gene [4]. Gene candidate approaches are currently underway to determine the role of these genes in the pathogenesis of psoriasis. While previous linkage studies were done with rather low-resolution genetic mapping tools, recent progress has led to the performance of whole genome scans using tightly spaced single nucleotide polymorphism (SNP) markers. This type of whole genome scan in combination with association studies for the first time provides a comprehensive perspective on gene variants contributing to disease risk. Already, interesting novel gene variants associated with critical immune pathways of complex genetic disorders have been detected, such as an IL-23R variant in inflammatory bowel disease [5]. This disease-protective gene variant is represented by a non-synonymous SNP in the IL-23R on chromosome 1p31 leading to an exchange of a glutamine for an arginine at position 381 of the IL-23R intracellular signalling chain. We and others have identified the same gene variant in psoriasis, implicating the general importance of the IL-23 pathway in chronic epithelial inflammation [6, 7]. It is of interest that there is overlap of susceptibility loci in psoriasis and atopic dermatitis, namely PSORS2, PSORS4 and PSORS5, indicating potentially shared biochemical pathways between the two major inflammatory skin diseases [8]. Whole genome analysis of genes is in line with analysis of gene transcripts in psoriasis. A recent study has identified 1,338 genes differentially expressed in psoriatic skin, the majority of them involved in immune response and proliferation [9]. Thus, both at the gene and transcriptome level, alterations in genes that are affecting the immune system are abundantly present in psoriasis.

Immune Effector Cells

A major role for the immune system in the pathogenesis of psoriasis is supported by the following arguments: (1) transfer or cure of psoriasis after bone marrow transplantation depending on the transplanted marrow and the recipient [10, 11]; (2) therapeutic activity of drugs targeting the immune system [12–15]; (3) expansion of clonal T cells in lesions over time [16]; (4) essential role of T cells/cytokines in humanized mouse models [17]; (5) possible genetic association with HLA-Cw6 [4, 18]. Immune cells with potential contribution to disease pathogenesis include T cells, dendritic cells (DC) and monocytes/macrophages [19–21]. CD8 T cells are mainly positioned in the epidermis in close contact to keratinocytes and Langerhans cells, while CD4 T helper (Th) cells are mainly located in the dermis with a preference for the upper papillary region. Substantial evidence points to an important role of epidermal T cells, including clonality of T cell receptors [22] and association of reduced numbers of epidermal T cells with therapeutic response [13]. Lesional psoriatic T cells are of an activated memory phenotype expressing CD25 [23] and secreting predominantly interferon (IFN)- γ . Thus, these cells might be classified as Th1 or T1 cells [24–26]. Recent interest has focused on Th17 cells producing IL-17 and a cytokine involved in epithelial hyperplasia (IL-22), but the functional role of Th17 cells in psoriasis awaits further clarification [27]. There is also a spectrum of so-called unconventional T cells or innate lymphocytes recognizing lipid antigens in the context of the non-polymorphic major histocompatibility complex class I-like protein CD1d. These natural killer T cells as well as CD1d-expressing keratinocytes and DC are present in psoriasis lesions potentially contributing to the inflammatory milieu [28, 29].

Antigen-presenting DC direct an immune response by activating and/or priming T cells and potentially other immune effectors such as B cells and natural killer cells. Dermal DC are increased in psoriasis lesions, and induce auto-proliferation and the production of Th1 cytokines in autologous T cells [26]. A subset of CD1d negative HLA-DR positive DC also induces increased autologous T cell proliferation [30]. Immune intervention using cyclosporin A or alefacept in psoriasis patients reduces DC numbers, further supporting their key role in psoriasis pathogenesis [31, 32]. Major subsets of DC include myeloid DC such as dermal DC or plasmacytoid DC (PDC). PDC producing IFN- α are increased in psoriasis lesions and blocking PDC or IFN- α will prevent psoriasis development in psoriasis models [33]. Thus, there is substantial scientific evidence that various subsets of DC are key activators of psoriatic T cells and potential therapeutic targets.

Under physiological circumstances there needs to be a balance between forces activating and suppressing the immune system. Therefore, an understanding

of immunosuppressive effector pathways and their potential dysregulation in autoimmunity is of paramount importance. Regulatory T cells suppress inflammatory processes either through cytokine production or cell/cell contact. While studies have indicated that regulatory T cell numbers are not altered in psoriasis, there seems to be a defect in their suppressive activity [34]. Other immunologically active cells in psoriasis lesions include monocyte/ macrophages, neutrophils and mast cells with a possible contribution from endothelial cells, fibroblasts and keratinocytes [35–37]. Recent support for a possible functional role of macrophages comes from studies using genetically modified mice [38–40]. These studies await confirmation from functional investigation of human psoriasis.

Cytokines

Immune cells communicate with each other by low molecular weight secreted molecules, so-called cytokines. The cytokine network hypothesis in psoriasis predicted a key role for pro-inflammatory cytokines including TNF- α and IL-8 [41]. Therapeutic success of anti-TNF- α therapy in the treatment of psoriasis has validated the cytokine network hypothesis and supports the search for other key effector cytokines as possible drug targets in psoriasis. IFN- γ and IFN- γ -induced gene products are central to the psoriatic cytokine network, however, blocking of IFN- γ has yet to demonstrate its therapeutic efficacy. The cytokine network hypothesis has been recently revisited and includes now novel cytokines such as IL-23 and IL-17 [42]. IL-23 belongs to the IL-12 family of cytokines and is increased in psoriatic lesion. A major source are IL-23-producing DC [43]. Blocking IL-12 cytokine family members including IL-23 using an anti-p40 monoclonal antibody has recently shown therapeutic benefit in a randomized phase II study [44]. However, due to the absence of an IL-23-specific monoclonal antibody tested in relevant functional studies, the functional role of IL-23 independent from IL-12 awaits further clarifications. IL-23 stimulates IL-17-producing Th17 cells. Th17 cells are also characterized by the production of IL-22. Interestingly, IL-22 induces epidermal hyperplasia and therefore might provide a potential link between IL-23 production, Th17 cells and epidermal hyperplasia in psoriasis. However, this sequence of pathological events is currently hypothetical and unproven in the case of psoriasis.

Psoriasis: An Autoimmune Disease?

Psoriasis qualifies as an autoimmune disease if one applies a definition proposed in a 2001 *New England Journal of Medicine* review article: ‘a clinical

Table 1. Arguments for an autoimmune aetiology of psoriasis

Chronic recurrent inflammation in the absence of an ongoing infection
Therapeutic response to immunointervention
Autostimulatory properties of lesional dendritic cells
Autoantibodies to skin products
Identical clonal T cells appear over time in psoriasis lesions and identical twins

syndrome caused by the activation of T cells or B cells, or both, in the absence of an ongoing infection or other discernible cause' [45]. There is some suggestive evidence in the literature pointing to the existence of a potential (auto-)antigen, however, no direct functional evidence has been provided for any of the candidate antigens. Autoreactivity conferred by psoriatic DC is suggested by the fact that lesional dendritic cells isolated from psoriatic lesions induce a highly increased autoreactive T cell proliferation and Th1 cytokine production [26]. Studies of autoantibodies in psoriasis have found reactivity against autoantigens, including keratin 13 and heterogeneous nuclear ribonucleoprotein-A1 [46]. Autoantibodies to antigens present in psoriatic keratinocytes include anti-HSP60 and anti-streptococcal reactivity [47]. In future studies, it will be important to compare frequencies of these autoantibodies (in psoriasis patients, healthy volunteers and patients with inflammatory skin disease other than psoriasis), as well as their potential functional role in psoriasis.

Evidence for a potential antigen-driven expansion of T cells in psoriasis comes mainly from molecular studies of T cell receptor clonality. The usage of T cell receptors in psoriasis seems to be highly restricted. The same T cell receptor clone was followed over time in evolving lesions and was present in lesions of identical twins [16, 48]. The most widely studied antigenic trigger for psoriasis is β -haemolytic streptococci. Psoriasis patients may be exposed to these bacteria as part of a streptococcal tonsillitis. While incidence rates of streptococcal infections preceding psoriasis have been reported as ranging from 60 to 97% [49, 50], the percentage of patients where streptococci can be cultured from tonsillar swabs is quite low [51]. It has been proposed that psoriasis is a T cell-mediated autoimmune disease induced by streptococcal superantigens [52]. However, psoriasis does not resemble the transitory skin rash of streptococcal superantigen-induced scarlet fever and is generally not responsive to antibiotic treatment. An alternative scenario of streptococci-induced psoriasis is related to the concept of molecular mimicry, which is based on the recognition of an infectious agent and a self antigen by the same T cell clone. In the case of streptococci it has been proposed that the T cells reactive to streptococcal

M proteins cross-react with keratins in human epidermis and thus lead to a sustained sterile (but originally antibacterial) inflammation [53]. A convincing molecular link for this hypothesis is provided by the finding of identical TCR clonality in streptococcal angina and skin lesions of patients with psoriasis [54]. In this context it is tempting to speculate that psoriasis is based on a sterile antibacterial immune response where a primarily anti-streptococcal immune response, potentially based on the activation and expansion of Th17 effector cells, is now directed against harmless constituents of skin.

Immunotherapy

Therapy of psoriasis has moved from serendipity to targeted intervention based on increased insight into its pathogenesis. These biological therapies take advantage of specific monoclonal antibodies or fusion proteins targeting specific immunological effectors. Current approaches can be broadly divided into therapeutic approaches blocking cytokines or targeting T cells.

The main target in anti-cytokine therapy is TNF- α . This is based on the success story of this type of treatment in rheumatoid disease [55]. Multiple fusion proteins and antibodies are currently on the market or are on their way to the market aiming at the blockade of TNF- α in the treatment of psoriasis [1].

Treatment of psoriasis patients with infliximab, a monoclonal antibody targeting TNF- α , was one of the first investigator-initiated proof of concept studies of the activity of biologicals in psoriasis [56]. Infliximab has multiple potential mechanisms of action, including neutralization of circulating trimeric TNF- α , generation of high molecular anti-TNF/TNF complexes with potential activity through Fc receptors and complement receptors, and binding to cell surface-bound TNF- α or to TNF- α bound to TNF receptors. Infliximab has one of the highest clinical response rates of current systemic anti-psoriatic treatments with a Psoriasis Area Severity Index (PASI) 75 response rate (often corresponding to a physician global assessment of clear to almost clear) of 80% at week 10 of treatment and prolonged treatment response until week 50 [57]. Potential side effects include immediate and delayed-type hypersensitivity infusion reactions, infections (including serious infections such as tuberculosis), hepatitis and in very rare cases potentially fatal hepatosplenic T cell lymphoma (mostly young adults with concomitant immunosuppression).

Etanercept is a p75 TNF receptor fusion protein which binds to soluble and membrane-bound TNF and lymph toxin. Etanercept is injected twice weekly through the subcutaneous route. Therapeutic response is dose related, with 34% and 49% of patients receiving 25 and 50 mg twice weekly, respectively, achieving >75% improvement in PASI 75 response after 12 weeks of treatment [58].

Safety issues reflect the overall immunosuppressive activity of anti-TNF- α agents and include few cases with demyelisation.

A novel anti-cytokine approach targets members of the IL-12 cytokine super family (IL-12 and IL-23). These are heterodimeric cytokines sharing a common p40 chain. An antibody targeting the common chain of IL-12/IL-23, that is IL-12p40 and IL-23p40, has demonstrated efficacy in a phase II trial with PASI 75 response at week 12 in 52% of patients who received a single 45-mg dose and in 59% of those who received a single 90-mg dose, compared with 2% of those who received placebo [44]. This impressive clinical efficacy after a single dose requires confirmation in future phase III trials as well as safety data in a higher number of patients, but suggests that another master cytokine in the treatment of psoriasis might be on the horizon [59].

T cell-targeted therapies include targets such as leukocyte function-associated antigen (LFA-3; LFA-3 fusion protein alefacept) and CD11a (anti-CD11a monoclonal antibody efalizumab).

Alefacept is the first biological therapy developed for psoriasis. A proof of concept study demonstrated that after a 12-week course of intramuscular alefacept (15 mg weekly), 33% of patients achieved at least a PASI 75 response at any time [60]. Alefacept is an LFA-3 fusion protein which blocks engagement of LFA-3 with its ligand CD2, for example on memory T cells. Additional mechanism of action relates to killing of alefacept-binding targets through antibody-dependent cell-mediated cytotoxicity. Alefacept depletes circulating memory T cells [61], but primary and secondary humoral immune responses are maintained [62].

Efalizumab is a humanized antibody binding to CD11a, the α -subunit of LFA-1. It inhibits its interaction with ICAM-1 (CD54). Its suspected mechanism of action ranges from blocking T cell/dendritic cell interaction in the skin to blocking entrance of immune cells into skin and a possible blockade of T cell/dendritic cell interaction in skin draining lymph nodes. Efalizumab is injected on a weekly basis subcutaneously typically over 12 weeks. A reduced starting dose is used to prevent an initial flu-like syndrome. Efalizumab has a reasonable clinical efficacy with a PASI 75 response rate of about 22% [60], which might be increasing over prolonged treatment periods. Long-term treatment over 27 months has demonstrated efficacy and safety in an open label study [63]. Efalizumab is generally well tolerated. Side effects include transient papular eruptions, initial transitory flu-like syndrome and rare cases of thrombocytopenia.

Pathogenesis-based and effective systemic therapies for patients with psoriasis are becoming a reality [64]. Long-term safety issues remain a potential concern. The first data based on well-controlled registries for rheumatological disorders did not show major new safety signals compared to other systemic treatment approaches [65].

Conclusion

Psoriasis ranks now among the major common autoimmune-type inflammatory disorders such as rheumatoid arthritis or inflammatory bowel disease. It often shares pathologic pathways and also therapeutic targets with these disorders. Future developments for research and therapy of psoriasis might include (1) a synergistic view between our genomic and immunologic understanding of the disease [3], (2) a focus on very early initiating events in the pathogenesis of psoriasis [33], (3) a focus on the systemic inflammatory components of psoriasis with impacts on the cardiovascular system [66, 67] and (4) a focus on the skin-specific tissue environment, the role of skin-resident immune effector cells and their interaction with the epithelium and the surrounding connective tissue [68].

References

- 1 Lowes MA, Bowcock AM, Krueger JG: Pathogenesis and therapy of psoriasis. *Nature* 2007;445:866–873.
- 2 von Hebra F: Acute Exantheme und Hautkrankheiten; in von Virchow R: *Handbuch der speciellen Pathologie und Therapie*. Erlangen, Verlag von Ferdinand Enk, 1860.
- 3 Bowcock AM, Krueger JG: Getting under the skin: the immunogenetics of psoriasis. *Nat Rev Immunol* 2005;5:699–711.
- 4 Capon F, Munro M, Barker J, Trembath R: Searching for the major histocompatibility complex psoriasis susceptibility gene. *J Invest Dermatol* 2002;118:745–751.
- 5 Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, et al: A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006;314:1461–1463.
- 6 Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP, Matsunami N, Ardlie KG, Civello D, Catanese JJ, et al: A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007;80:273–290.
- 7 Capon F, Di Meglio P, Szaub J, Prescott NJ, Dunster C, Baumber I, Timms K, Gutin A, Abkevich V, Burden AD, Launchbury J, Barker JN, Trembath RC, Nestle FO: Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. *Hum Genet* 2007;122:201–206.
- 8 Cookson WO, Ubhi B, Lawrence R, Abecasis GR, Walley AJ, Cox HE, Coleman R, Leaves NI, Trembath RC, Moffatt MF, et al: Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nat Genet* 2001;27:372–373.
- 9 Zhou X, Krueger JG, Kao MC, Lee E, Du F, Menter A, Wong WH, Bowcock AM: Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* 2003;13:69–78.
- 10 Eedy DJ, Burrows D, Bridges JM, Jones FG: Clearance of severe psoriasis after allogeneic bone marrow transplantation. *BMJ* 1990;300:908.
- 11 Gardembas-Pain M, Ifrah N, Foussard C, Boasson M, Saint Andre JP, Verret JL: Psoriasis after allogeneic bone marrow transplantation. *Arch Dermatol* 1990;126:1523.
- 12 Ellis CN, Gorsulowsky DC, Hamilton TA, Billings JK, Brown MD, Headington JT, Cooper KD, Baadsgaard O, Duell EA, Annesley TM, et al: Cyclosporine improves psoriasis in a double-blind study. *JAMA* 1986;256:3110–3116.

- 13 Gottlieb SL, Gilleaudeau P, Johnson R, Estes L, Woodworth TG, Gottlieb AB, Krueger JG: Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat Med* 1995;1:442–447.
- 14 Prinz J, Braun-Falco O, Meurer M, Daddona P, Reiter C, Rieber P, Riethmuller G: Chimaeric CD4 monoclonal antibody in treatment of generalised pustular psoriasis. *Lancet* 1991;338:320–321.
- 15 Bachelez H, Flageul B, Dubertret L, Fraitag S, Grossman R, Brousse N, Poisson D, Knowles RW, Wacholtz MC, Haverty TP, et al: Treatment of recalcitrant plaque psoriasis with a humanized non-depleting antibody to CD4. *J Autoimmun* 1998;11:53–62.
- 16 Menssen A, Trommler P, Vollmer S, Schendel D, Albert E, Gurtler L, Riethmuller G, Prinz JC: Evidence for an antigen-specific cellular immune response in skin lesions of patients with psoriasis vulgaris. *J Immunol* 1995;155:4078–4083.
- 17 Nestle FO, Nickoloff BJ: From classical mouse models of psoriasis to a spontaneous xenograft model featuring use of AGR mice. *Ernst Schering Res Found Workshop* 2005;203–212.
- 18 Nair RP, Stuart PE, Nistor I, Hiremagalore R, Chia NV, Jenisch S, Weichenthal M, Abecasis GR, Lim HW, Christophers E, et al: Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *Am J Hum Genet* 2006;78:827–851.
- 19 Braun-Falco O, Burg G: Inflammatory infiltrate in psoriasis vulgaris: a cytochemical study. *Arch Klin Exp Dermatol* 1970;236:297–314.
- 20 Bos JD, Hulsebosch HJ, Krieg SR, Bakker PM, Cormane RH: Immunocompetent cells in psoriasis: in situ immunophenotyping by monoclonal antibodies. *Arch Dermatol Res* 1983;275:181–189.
- 21 Baker BS, Swain AF, Fry L, Valdimarsson H: Epidermal T lymphocytes and HLA-DR expression in psoriasis. *Br J Dermatol* 1984;110:555–564.
- 22 Chang JC, Smith LR, Froning KJ, Schwabe BJ, Laxer JA, Caralli LL, Kurland HH, Karasek MA, Wilkinson DI, Carlo DJ, et al: CD8+ T cells in psoriatic lesions preferentially use T-cell receptor V β 3 and/or V β 13.1 genes. *Proc Natl Acad Sci USA* 1994;91:9282–9286.
- 23 Gottlieb AB, Lifshitz B, Fu SM, Staiano-Coico L, Wang CY, Carter DM: Expression of HLA-DR molecules by keratinocytes, and presence of Langerhans cells in the dermal infiltrate of active psoriatic plaques. *J Exp Med* 1986;164:1013–1028.
- 24 Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ: The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. *J Invest Dermatol* 1993;101:701–705.
- 25 Schlaak JF, Buslau M, Jochum W, Hermann E, Girndt M, Gallati H, Meyer zum Buschenfelde KH, Fleischer B: T cells involved in psoriasis vulgaris belong to the Th1 subset. *J Invest Dermatol* 1994;102:145–149.
- 26 Nestle FO, Turka LA, Nickoloff BJ: Characterization of dermal dendritic cells in psoriasis: autostimulation of T lymphocytes and induction of Th1 type cytokines. *J Clin Invest* 1994;94:202–209.
- 27 Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, Ouyang W: Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 2007;445:648–651.
- 28 Bonish B, Jullien D, Dutronc Y, Huang BB, Modlin R, Spada FM, Porcelli SA, Nickoloff BJ: Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN- γ production by NK-T cells. *J Immunol* 2000;165:4076–4085.
- 29 Gerlini G, Hefti HP, Kleinhaus M, Nickoloff BJ, Burg G, Nestle FO: Cd1d is expressed on dermal dendritic cells and monocyte-derived dendritic cells. *J Invest Dermatol* 2001;117:576–582.
- 30 Baadsgaard O, Gupta AK, Taylor RS, Ellis CN, Voorhees JJ, Cooper KD: Psoriatic epidermal cells demonstrate increased numbers and function of non-Langerhans antigen-presenting cells. *J Invest Dermatol* 1989;92:190–195.
- 31 Baker BS, Griffiths CE, Lambert S, Powles AV, Leonard JN, Valdimarsson H, Fry L: The effects of cyclosporin A on T lymphocyte and dendritic cell sub-populations in psoriasis. *Br J Dermatol* 1987;116:503–510.
- 32 Chaman F, Lowes MA, Lin SL, Lee E, Kikuchi T, Gilleaudeau P, Sullivan-Whalen M, Cardinale I, Khatcherian A, Novitskaya I, et al: Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. *Proc Natl Acad Sci USA* 2005;102:2075–2080.

- 33 Nestle FO, Conrad C, Tun-Kyi A, Homey B, Gombert M, Boyman O, Burg G, Liu YJ, Gilliet M: Plasmacytoid dendritic cells initiate psoriasis through interferon- α production. *J Exp Med* 2005;202:135–143.
- 34 Sugiyama H, Gyulai R, Toichi E, Garacz E, Shimada S, Stevens SR, McCormick TS, Cooper KD: Dysfunctional blood and target tissue CD4+CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. *J Immunol* 2005;174:164–173.
- 35 Nickoloff BJ, Mitra RS, Green J, Zheng XG, Shimizu Y, Thompson C, Turka LA: Accessory cell function of keratinocytes for superantigens: dependence on lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction. *J Immunol* 1993;150:2148–2159.
- 36 Pober JS, Kluger MS, Schechner JS: Human endothelial cell presentation of antigen and the homing of memory/effector T cells to skin. *Ann NY Acad Sci* 2001;941:12–25.
- 37 Filer A, Raza K, Salmon M, Buckley CD: Targeting stromal cells in chronic inflammation. *Discov Med* 2007;7:20–26.
- 38 Wang H, Peters T, Kess D, Sindrilaru A, Oreshkova T, van Rooijen N, Stratis A, Renkl AC, Sunderkotter C, Wlaschek M, et al: Activated macrophages are essential in a murine model for T cell-mediated chronic psoriasiform skin inflammation. *J Clin Invest* 2006;116:2105–2114.
- 39 Stratis A, Pasparakis M, Rupec RA, Markur D, Hartmann K, Scharffetter-Kochanek K, Peters T, van Rooijen N, Krieg T, Haase I: Pathogenic role for skin macrophages in a mouse model of keratinocyte-induced psoriasis-like skin inflammation. *J Clin Invest* 2006;116:2094–2104.
- 40 Clark RA, Kupper TS: Misbehaving macrophages in the pathogenesis of psoriasis. *J Clin Invest* 2006;116:2084–2087.
- 41 Nickoloff BJ, Karabin GD, Barker JN, Griffiths CE, Sarma V, Mitra RS, Elder JT, Kunkel SL, Dixit VM: Cellular localization of interleukin-8 and its inducer, tumor necrosis factor- α in psoriasis. *Am J Pathol* 1991;138:129–140.
- 42 Nickoloff BJ: Cracking the cytokine code in psoriasis. *Nat Med* 2007;13:242–244.
- 43 Lee E, Trepicchio WL, Oestreicher JL, Pittman D, Wang F, Chamian F, Dhodapkar M, Krueger JG: Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J Exp Med* 2004;199:125–130.
- 44 Krueger GG, Langley RG, Leonardi C, Yeilding N, Guzzo C, Wang Y, Dooley LT, Lebwohl M: A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. *N Engl J Med* 2007;356:580–592.
- 45 Davidson A, Diamond B: Autoimmune diseases. *N Engl J Med* 2001;345:340–350.
- 46 Jones DA, Yawalkar N, Suh KY, Sadat S, Rich B, Kupper TS: Identification of autoantigens in psoriatic plaques using expression cloning. *J Invest Dermatol* 2004;123:93–100.
- 47 Cancino-Diaz ME, Ruiz-Gonzalez V, Ramirez-Resendiz L, Ortiz B, Dominguez-Lopez ML, Paredes-Cabrera GC, Leon-Dorantes G, Blancas-Gonzalez F, Jimenez-Zamudio L, Garcia-Latorre E: IgG class antibodies from psoriasis patients recognize the 60-KDa heat-shock protein of *Streptococcus pyogenes*. *Int J Dermatol* 2004;43:341–347.
- 48 Prinz JC, Vollmer S, Boehncke WH, Menssen A, Laisney I, Trommler P: Selection of conserved TCR VDJ rearrangements in chronic psoriatic plaques indicates a common antigen in psoriasis vulgaris. *Eur J Immunol* 1999;29:3360–3368.
- 49 Whyte HJ, Baughman RD: Acute guttate psoriasis and streptococcal infection. *Arch Dermatol* 1964;89:350–356.
- 50 Prinz J: Psoriasis; in Bos J (ed): *Skin Immune System*. Boca Raton, CRC Press, 2004, pp 615–626.
- 51 Gudjonsson JE, Thorarinsson AM, Sigurgeirsson B, Kristinsson KG, Valdimarsson H: Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. *Br J Dermatol* 2003;149:530–534.
- 52 Valdimarsson H, Baker BS, Jonsdottir I, Powles A, Fry L: Psoriasis: a T-cell-mediated autoimmune disease induced by streptococcal superantigens? *Immunol Today* 1995;16:145–149.
- 53 Gudmundsdottir AS, Sigmundsdottir H, Sigurgeirsson B, Good MF, Valdimarsson H, Jonsdottir I: Is an epitope on keratin 17 a major target for autoreactive T lymphocytes in psoriasis? *Clin Exp Immunol* 1999;117:580–586.

- 54 Diluvio L, Vollmer S, Besgen P, Ellwart JW, Chimenti S, Prinz JC: Identical TCR β -chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. *J Immunol* 2006;176:7104–7111.
- 55 Feldmann M, Maini RN: Anti-TNF- α therapy of rheumatoid arthritis: what have we learned? *Annu Rev Immunol* 2001;19:163–196.
- 56 Chaudhari U, Romano P, Mulcahy LD, Dooley LT, Baker DG, Gottlieb AB: Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet* 2001;357:1842–1847.
- 57 Reich K, Nestle FO, Papp K, Ortonne JP, Evans R, Guzzo C, Li S, Dooley LT, Griffiths CE: Infliximab induction and maintenance therapy for moderate-to-severe psoriasis: a phase III, multicentre, double-blind trial. *Lancet* 2005;366:1367–1374.
- 58 Leonardi CL, Powers JL, Matheson RT, Goffe BS, Zitnik R, Wang A, Gottlieb AB: Etanercept as monotherapy in patients with psoriasis. *N Engl J Med* 2003;349:2014–2022.
- 59 Nestle FO, Conrad C: The IL-12 family member p40 chain as a master switch and novel therapeutic target in psoriasis. *J Invest Dermatol* 2004;123:xiv–xv.
- 60 Lebwohl M, Tying SK, Hamilton TK, Toth D, Glazer S, Tawfik NH, Walicke P, Dummer W, Wang X, Garovoy MR, et al: A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *N Engl J Med* 2003;349:2004–2013.
- 61 Ellis CN, Krueger GG: Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. *N Engl J Med* 2001;345:248–255.
- 62 Gottlieb AB, Casale TB, Frankel E, Goffe B, Lowe N, Ochs HD, Roberts JL, Washenik K, Vaishnav AK, Gordon KB: CD4+ T-cell-directed antibody responses are maintained in patients with psoriasis receiving alefacept: results of a randomized study. *J Am Acad Dermatol* 2003;49:816–825.
- 63 Gottlieb AB, Hamilton T, Caro I, Kwon P, Compton PG, Leonardi CL: Long-term continuous efalizumab therapy in patients with moderate to severe chronic plaque psoriasis: updated results from an ongoing trial. *J Am Acad Dermatol* 2006;54:S154–S163.
- 64 Smith CH, Barker JN: Psoriasis and its management. *BMJ* 2006;333:380–384.
- 65 Dixon WG, Watson K, Lunt M, Hyrich KL, Silman AJ, Symmons DP: Rates of serious infection, including site-specific and bacterial intracellular infection, in rheumatoid arthritis patients receiving anti-tumor necrosis factor therapy: results from the British Society for Rheumatology Biologics Register. *Arthritis Rheum* 2006;54:2368–2376.
- 66 Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB: Risk of myocardial infarction in patients with psoriasis. *JAMA* 2006;296:1735–1741.
- 67 Sommer DM, Jenisch S, Suchan M, Christophers E, Weichenthal M: Increased prevalence of the metabolic syndrome in patients with moderate to severe psoriasis. *Arch Dermatol Res* 2006;298:321–328.
- 68 Boyman O, Conrad C, Tonel G, Gillet M, Nestle FO: The pathogenic role of tissue-resident immune cells in psoriasis. *Trends Immunol* 2007;28:51–57.

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Atopic Dermatitis in 2008

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Abstract

Atopic dermatitis (also termed atopic eczema and infantile eczema), a chronic, itchy, inflammatory skin disease that sets on at infancy or early childhood, is observed with increasing prevalence around the world, particularly in developed nations. Although sufficient evidences are not yet available to define it as a classical autoimmune disease, autoantigens have been identified. Investigations of atopic dermatitis in human patients and animal models suggest that this disease is initiated, maintained and perpetuated by the actions of cytokines, chemokines, T cells, antigen-presenting cells and other inflammatory cells; there is also evidence of skin barrier defect and angiogenesis. Recent identification of mutations of the epidermal barrier protein filaggrin (encoded by *FLG*), present in about 9% of people of European origin, with 70% of individuals homozygous or compound heterozygous for *FLG* null alleles developing atopic dermatitis, provides a strong link between a defect of the epidermal barrier that allows easy penetration of pathogen/allergen through the skin and a systemic hyperactive immune response to the penetrated pathogen/allergen. The newly introduced concept of ‘intrinsic’ and ‘extrinsic’ atopic dermatitis has fueled the debate among academic dermatologists as to how ‘atopic’ atopic dermatitis should be defined. Some recent advancements on the management options for atopic dermatitis are also discussed.

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What is Atopic Dermatitis? Background and Definition

Atopy, an English term derived from the Greek word ‘ατοπος’, which carries an original meaning of ‘out of place’ or ‘strange disease’, was first introduced to the biomedical community by Coca and Cooke in 1923, in describing the disease phenomena of hypersensitivity in nature [1, 2]. In their paper, the definition of ‘atopy’ includes only asthma and allergic rhinitis, but not atopic skin disease [1, 2]. Perhaps the earliest academicians who coined the term of ‘atopic dermatitis’ were Wise and Sulzberger in 1933 [1, 3]. In their early description of atopic dermatitis, Wise and Sulzberger defined atopic dermatitis

to include those disease entities then recognized as ‘generalized neurodermatitis’ and ‘diffuse pruritus with lichenification’, characterized by 9 cardinal qualities: (1) atopic family history; (2) antecedent infantile eczema; (3) localization of lesions in antecubital and popliteal fossae, anterior neck, chest, face, and eyelids; (4) grayish or brownish discoloration; (5) lack of true clinical or histological vesicles; (6) vasomotor instability or irritability; (7) negative patch test to contact allergens; (8) positive reactions of immediate wheal type to scratch or intradermal testing; (9) positive serum regains [1, 3]. Subsequent long-term studies of infant-onset atopic dermatitis (infantile eczema) confirmed the clinical association between atopic dermatitis and asthma and allergic rhinitis [1, 4, 5]. The most commonly referred definition of atopic dermatitis is likely the one derived by Hanifin and Rajka [6] in 1980. In this definition, atopic dermatitis must fulfill at least 3 major and 3 minor criteria in a rather complex list of findings (table 1). In an international consensus conference on atopic dermatitis conducted in Rome, Italy, in 1999, some experts expressed concern that this 1980s definition may be too complicated to be used for large-scale epidemiological studies and that some of the minor criteria listed may not be sufficiently specific to be included [7]. In fact, some of the European investigators started using a simplified set of criteria [7, 8]. In a United Kingdom refined set of diagnostic criteria, now widely used in more than 30 countries, a new definition of satisfaction of one major criterion (pruritic skin condition) plus 3 of the 5 minor criteria was established for the purpose of epidemiological studies (table 2) [8]. The most recent definition of atopic dermatitis (in children) was published in 2003 as a result of a consensus conference convened by the American Academy of Dermatology in 2001 and attended by a total of 40 academic dermatologists, with a newly revised set of criteria (table 3) [9]. In this 2003 definition, all cases of pediatric atopic dermatitis must have at least 2 of the following features: (1) pruritus and (2) eczema of acute, subacute or chronic stage, with typical morphology and age-specific patterns and with chronic or relapsing history (table 3) [9].

From the perspective of epidemiology, atopic dermatitis seems to have increased in prevalence over the past few decades, particularly in the developed nations in different parts of the world [10]. As for the terminology, many experts in the field concurred that atopic dermatitis is synonymous with atopic eczema and infantile eczema [1, 7, 10, 11]. In this chapter, we will exclusively use the term atopic dermatitis, for the sake of clarity and uniformity. In the last few years, the studies of atopic dermatitis have been further complicated by a newly proposed concept that there may be 2 different subsets of atopic dermatitis, with the ‘intrinsic’ (nonallergic) subset characterized by the absence of high level of total serum IgE and the ‘extrinsic’ (allergic) subset characterized by high serum IgE [11–21]. Just how ‘atopic’ should atopic dermatitis be defined and can high

Table 1. The 1980s diagnostic criteria for atopic dermatitis

Major criteria (need minimum 3 of the following 4 features for the diagnosis)
Pruritus
Typical morphology and distribution
Flexural lichenification or linearity in adults
Facial and extensor involvement in infants and children
Chronic or chronically relapsing dermatitis
Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis)
Minor criteria (need minimum 3 of the following features for the diagnosis)
Xerosis
Ichthyosis/palmar linearity/keratosis pilaris
Immediate (type I) skin test reactivity
Elevated serum IgE
Early age of onset
Tendency toward cutaneous infections/impaired cell-mediated immunity
Tendency toward nonspecific hand or foot dermatitis
Nipple eczema
Cheilitis
Recurrent conjunctivitis
Dennie-Morgan infraorbital fold
Keratoconus
Anterior subcapsular cataracts
Orbital darkening
Facial pallor/facial erythema
Pityriasis alba
Anterior neck folds
Itch when sweating
Intolerance to wool and lipid solvents
Perifollicular accentuation
Food intolerance
Course influenced by environmental/emotional factors
White dermatographism/delayed blanch

| Derived from Hanifin and Rajka [6]. Both major and minor criteria are needed for the diagnosis. |

serum IgE, a criterion that defines atopy, be excluded from the definition of atopic dermatitis [11]? This is indeed an active debate among academic dermatologists and will need a clearer answer in the future. So what is atopic dermatitis? The final verdict is not yet decided. The prevailing view is that atopic dermatitis is best viewed as a chronic, pruritic, inflammatory skin syndrome, rather than a disease or a condition and that it may or may not be associated with

Table 2. The United Kingdom's refined diagnostic criteria of atopic dermatitis for epidemiologic studies

Major Criteria (must be present for the diagnosis)
 Any pruritic skin condition in the past 12 months
 Minor Criteria (3 of the following must be present)
 Age of onset younger than 2 years¹
 History of flexural skin involvement
 History of generalized dry skin
 Personal history of other atopic disease²
 Clinical flexural dermatitis documented by photographic protocol

Derived from Williams [7].

¹Not used for children younger than 4 years.

²For children younger than 4 years, history of atopic disease in a first-degree relative may be used.

Table 3. Clinical diagnostic criteria for pediatric atopic dermatitis

Essential features (must be present for the diagnosis)
 Pruritus
 Eczema (acute, subacute, or chronic stage)
 Typical morphology and age-specific patterns (face/neck/extensor for infant and children, flexure in any age, sparing groin/axilla)
 Chronic or relapsing history
 Important features (observed in most cases and supported the diagnosis)
 Early onset age
 Atopy
 Personal and/or family history
 IgE reactivity
 Xerosis
 Associate features (suggested the diagnosis but nonspecifically)
 Atypical vascular responses (such as facial pallor, delayed blanch response, white dermatographism)
 Keratosis pilaris/palmar hyperlinearity/ichthyosis
 Ocular/periorbital lesions
 Perifollicular accentuation/lichenification/prurigos
 Perioral/periauricular lesions

Derived from Eichenfield et al. [9]. The diagnosis of atopic dermatitis is also dependent on the exclusion of the following conditions: scabies, seborrheic dermatitis, allergic contact dermatitis, ichthyoses (except vulgaris subtype), cutaneous lymphoma, psoriasis and immune deficiency diseases.

asthma or allergic rhinitis [1, 9]. Based on the currently available data from human patient studies and animal models of atopic dermatitis, it is best to view that atopic dermatitis may be initiated by a combination of epidermal barrier defects and immune hyperreaction to allergens, consisting of an initial imbalance of cytokines favoring the helper T cell (Th) subset 2 type, maintained by cytokines of the Th1 type, and perpetuated by an autoimmune process, along with the participation of chemokines, adhesion molecules, proangiogenic factors, T cells, antigen-presenting cells, mast cells and other inflammatory cells [10]. Some of these topics will be elaborated in the following sections.

The Immunological Basis of Disease Pathogenesis

Data from Human Patient Studies

Before discussing the immunological basis of human atopic dermatitis pathogenesis, one should be reminded to be cautious in interpreting the data from most previous studies. This precaution is needed, because most of these studies were conducted at the time when all cases of atopic dermatitis were presumed to be mediated by IgE, in other word before the concept of intrinsic and extrinsic subsets of atopic dermatitis was introduced to the dermatology community. So if the newly introduced intrinsic (non-IgE-mediated) subset of atopic dermatitis, characterized by the absence of high level of total serum IgE, is proven to be mediated by a fundamentally different immunological mechanism than the extrinsic (IgE-mediated) subset, the data obtained from previous studies may be applicable only to one or the other subset of atopic dermatitis, or possibly invalidated, depending on whether IgE levels were measured as a part of the study protocol in each of those studies. Additional caution in interpreting the data from human patient studies is warranted, as the findings are usually static in nature, because in vivo functional studies or step-by-step examinations of immune and clinical parameters of the disease's natural course (that is without medical treatment) are not allowed to be performed in human patients due to obvious ethical reasons.

Role of T Cells

T cells have long been considered participants of the atopic dermatitis pathogenesis due to their constant presence in both the acute and chronic stages of the disease. This hypothesis is based on the observations that T cells are detected in the lesional skin where they infiltrate both the epidermis and dermis [22–24], that most of the circulating and lesional T cells expressed skin-homing cutaneous lymphocyte-associated antigen (CLA) [25–27], that circulating and skin-infiltrating T cells from these patients are allergen reactive [25, 28], and

that the CLA⁺ T cells in patients with atopic dermatitis are highly activated [27]. All of these data together provide strong but indirect evidences that T cells indeed participate in the pathogenesis of atopic dermatitis in some ways.

Role of Antigen-Presenting Cells

A corollary of the T cell involvement hypothesis is that antigen-presenting cells are also involved, since a T cell-activating process generally requires the presentation of antigen from antigen-presenting cells [29]. In support of the participation of antigen-presenting cells are the findings that antigen-presenting cells are increasingly observed in lesional atopic dermatitis skin [22, 23], that circulating and skin-infiltrating T cells from these patients are allergen reactive [25, 28], and that antigen-presenting cells in atopic dermatitis patients are highly activated and show their ability to increase autologous T lymphocyte reactivity to lesional epidermal cells under laboratory experimental conditions [30].

Role of Cytokines

The early findings of elevation of total serum IgE in patients with atopic dermatitis naturally suggest that Th2-type cytokines may be participants of the disease pathogenesis, since IL-4, a critical Th2-type cytokine, must be present to convert B cells to IgE-producing cells, as IL-4 knockout mice were not able to generate detectable levels of IgE [31]. In fact, early studies of atopic dermatitis patients' skin revealed upregulation of Th2-type cytokine expressions. Subsequently, Th1-type cytokine IFN- γ was also detected in the skin lesions of atopic dermatitis [32]. To be specific, some studies showed high frequency of IL-4-producing CD4⁺ allergen-reactive T cells in atopic dermatitis lesional skin and predominance of production of IL-4 by circulating mononuclear cells in atopic dermatitis patients [28, 33, 34]. Other studies showed the circulating CLA⁺ skin-homing T cells in atopic dermatitis patients release an IL-13-dominated Th2 cytokine profile including IL-4, but also higher level of IFN- γ [27]. The increased IL-13 cytokine may also contribute to the enhanced IgE production known to occur in atopic dermatitis patients [27]. In pediatric atopic dermatitis patients, spontaneous expression of IL-4 mRNA in their peripheral blood lymphocytes was reported, supporting a notion of *in vivo* activation of these lymphocytes [35]. In addition, IL-10 was reported to be overexpressed in the skin lesions of atopic dermatitis [36]. Since IL-10 is a known inhibitor for cell-mediated immunity to bacterial pathogens and an inducer for mast cell growth, its increase in the lesional skin may account for the prominent mast cell infiltration in atopic dermatitis lesion [37] and for the propensity for atopic dermatitis patients to develop staphylococcal skin infection (table 1) [6]. Other cytokines that have been reported to be upregulated in atopic dermatitis patients include IL-18 and IL-31 [38–40]. Specifically, investigators found that the

quantity of IL-18 in the supernatants of peripheral mononuclear cells of patients with atopic dermatitis was significantly higher than that of normal individuals, when activated by *Staphylococcus aureus* enterotoxin B, that the level of active IL-18 in the sera of atopic dermatitis patients was upregulated at the exacerbation of their disease, and that there are significant associations of single nucleotide polymorphisms in exon 1, promoter region 1 and promoter region 2 with atopic dermatitis [38]. IL-31 is a T cell-derived cytokine that can induce pruritus [39, 40]. Recently, investigators have reported that by quantitative real-time PCR performed on reverse-transcribed RNA obtained from skin of atopic dermatitis patients the IL-31 mRNA levels were significantly upregulated in pruritic atopic skin specimens compared to nonpruritic psoriatic skin, that staphylococcal superantigen rapidly induced IL-31 expression in atopic dermatitis patients in vivo, that staphylococcal enterotoxin B induced IL-31 in leukocytes in vitro, and that the highest concentrations of IL-31 receptors were localized to dorsal root ganglia, the presumed cell body site of cutaneous sensory neurons [39]. Other investigators have found by quantitative real-time PCR performed on reverse-transcribed RNA obtained from skin of atopic dermatitis patients that IL-31 mRNA levels were significantly upregulated in atopic skin specimens, in comparison to normal individuals, and that the upregulation of IL-31 is paralleled with the increased mRNA levels of IL-4 and IL-13 in the skin, but not with IFN- γ [40].

Role of IgE

Consistent with enhanced IL-4 production as documented by the findings of high frequency of IL-4-producing CD4⁺ allergen-reactive T cells in atopic dermatitis lesional skin, predominance of production of IL-4 by circulating mononuclear cells in atopic dermatitis patients, and spontaneous expression of IL-4 mRNA in the peripheral blood lymphocytes of pediatric atopic dermatitis patients [28, 33–35], total serum IgE in atopic dermatitis patients is generally increased in vivo and in vitro [41, 42]. Since most of those serum IgE studies were performed before the concept of intrinsic atopic dermatitis was introduced, the normal total serum IgE levels found in the past would likely to have been taken from some of those intrinsic atopic dermatitis patients. In some studies, the levels of total serum IgE were correlated with the disease severity of atopic dermatitis [43]. Besides the well-known role of IgE in inducing mast cell degranulation that would contribute to the inflammatory reaction in atopic dermatitis, serum IgE in atopic dermatitis patients also participates in the activation of T lymphocytes in an allergen-specific manner [41]. Thus, the roles of IgE in atopic dermatitis may be both one of direct involvement in the release of inflammatory mediators and one of indirect involvement in the activation of T cells.

Role of Chemokines and Adhesion Molecules

For the inflammatory cells to infiltrate into the skin and cause inflammatory reaction, they must be recruited by the actions of some locally produced chemotactic factors present in the skin [44, 45]. In addition, the inflammatory cells that infiltrate into the skin's affected sites must travel through blood vessels and need the assistance of microvascular endothelial cells by way of adhesion molecules [44, 45].

Even in normal-appearing skin of patients with atopic dermatitis, the expressions of 4 endothelial cell adhesion molecules – vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin and P-selectin – were found to be increased in the dermal microvasculature, in comparison to normal nonatopic individuals [46, 47]. In lesional skin, the expressions of these adhesion molecules were even more pronounced, concomitant with the increase in the number of tumor necrosis factor (TNF)- α -containing cells, consistent with the known function of TNF- α as a potent upregulator of endothelial cell adhesion molecules [47]. These findings were subsequently confirmed by additional investigators [48]. Most recently, other investigators have found an increased expression of yet another endothelial cell adhesion molecule – vascular adhesion protein-1, an adhesion molecule with an enzymatic activity which partakes in the migration process of lymphocytes – in the dermal microvascular endothelial cells of atopic patients, compared to normal individuals [49]. The latter finding provides additional support for the role of adhesion molecules in aiding the inflammatory process in atopic dermatitis and for the role of lymphocytes. Furthermore, another adhesion molecule, integrin α 6, has also been found to be upregulated in expression in both the dermal microvascular endothelial cells and the epidermis [50]. The association of increased basal epidermal integrin α 6 expression with T cell influx into the epidermis suggests a role of this integrin in leading to an epidermotropism of T cells during the inflammatory process [50].

With regard to the chemokines, an extensive network of these chemoattractants has been suggested to play a role in the pathogenesis of atopic dermatitis, including primarily the CC chemokines CCL27, CCL17, CCL22, CCL18, CCL11, CCL13, CCL20 and CCL1, as well as others [51–66]. This subset of chemokines, as well as their corresponding chemokine receptors are upregulated in the atopic dermatitis skin, and likely involve the recruitment of leukocyte subsets of T cells, eosinophils and dendritic cells [51]. Eotaxin (CCL11), a CC chemokine and potent chemoattractant and activator of human eosinophils, basophils and Th2 lymphocytes, and its receptor CCR3, were reported to be significantly increased in both the levels of mRNA and protein, in the lesional skin of atopic dermatitis [52]. The mRNA levels of eotaxin and CCR3 were also increased in the normal-appearing skin of atopic dermatitis patients, suggesting

their roles in the initiation of the clinical inflammation [52]. Subsequently, other investigators also confirmed the increase in eotaxin mRNA level in both the acute and chronic lesional skin of atopic dermatitis patients, along with an increase in monocyte chemotactic protein-4 (MCP-4, CCL13), by in situ hybridization [53]. The correlation of the expressions of eotaxin and MCP-4 mRNA levels (in the number of positive cells) with disease severity and the presence of eosinophils and macrophages support these chemokines' roles in recruiting inflammatory cells into the inflamed skin site [53]. Since thymus- and activation-regulated chemokine (TARC, CCL17) is a Th2-selective chemoattractant, many investigators have looked into its possible role in atopic dermatitis [54–57]. CCL17, produced by epidermal keratinocytes, has been found to be expressed in the epidermis of lesional but not nonlesional atopic dermatitis skin, correlated with an upregulation of the proportion of CLA+ skin-homing lymphocytes that are also positive for CCR4, the receptor for CCL17 [54]. Subsequently, the upregulation of CCL17 was confirmed by another group of investigators on the plasma level, along with an increase in macrophage-derived chemokine (MDC, CCL22), another Th2-selective chemokine [55]. Importantly, they found the plasma levels of CCL17 and CCL22 were strongly correlated with disease severity [55]. Moreover, the mRNA levels of both chemokines were induced in primary epidermal keratinocytes upon stimulation by IFN- γ , suggesting a role of both these chemokines and IFN- γ in atopic dermatitis [55]. Other studies also supported the findings of upregulation of CCL17 and CCL22 in atopic dermatitis, but showed that not only Th2-selective chemokines like CCL17 and CCL22 [56], but also the Th1-selective chemokine Mig (monokine induced by IFN- γ), are upregulated in atopic dermatitis, suggesting that the disease process of atopic dermatitis not only involves the Th2 arm of the immune system, but the Th1 arm as well [57]. Another skin-selective chemokine that has been linked to atopic dermatitis is cutaneous T cell-attracting chemokine (CTACK, CCL27), which has attracted the attention of many investigators. Expressed predominantly by epidermal keratinocytes of the lesional skin of atopic dermatitis, CCL27 has been shown to increase significantly in the sera of atopic dermatitis patients and its serum levels correlated well with disease severity, serum soluble E-selectin levels, serum MDC levels and serum TARC levels [58]. This increase in serum level of CCL27 in atopic dermatitis has been confirmed by other groups and has been suggested as a disease marker as well [59, 60]. CCL18, a human chemokine secreted by monocytes and dendritic cells, has also been investigated for its potential role in atopic dermatitis [61]. It was found that CCL18 expressed by antigen-presenting cells in the dermis and by Langerhans and dendritic cells in the epidermis of atopic dermatitis skin, but not in normal or psoriatic skin, corresponds to the increased serum level of CCL18 [61]. Together with the finding that CCL18 binds to CLA+ skin-homing

T cells derived from atopic dermatitis individuals and induces this memory T cell migration in vitro, these data support a role of CCL18 in mediating skin homing of a population of memory T cells [61]. Another CC chemokine thought to be involved in atopic dermatitis is CCL20 (LARC), supported by the evidences that CCL20 (a keratinocyte-produced protein) is upregulated in the skin of atopic dermatitis lesion, that many skin-infiltrating inflammatory cells expressed the CCL20 receptor CCR6, and that CCL20 mRNA and protein in primary human keratinocytes are induced by the proinflammatory cytokines IL-1 and TNF- α which has been found to increase in the skin lesions of atopic dermatitis [62]. Furthermore, CCL1 (I-309), a CC chemokine produced by dendritic cells, mast cells and dermal endothelial cells, has also been examined for its potential role in atopic dermatitis [63]. The findings that CCL1 serum levels in atopic dermatitis patients are significantly higher than normal individuals, that the skin expression of CCL1 is significantly and selectively upregulated in atopic dermatitis in comparison to normal skin and other inflammatory skin diseases like psoriasis and cutaneous lupus, that cross-linking of IgE on mast cells resulted in significant upregulation of CCL1, and that the CCL1 receptor CCR8 is expressed in a subset of T cells, provide a supporting argument for the role of CCL1 in atopic dermatitis [63]. Besides eotaxin (CCL11), another subspecies of eotaxin (eotaxin-3, CCL26) has also been reported to play a role in atopic dermatitis [64, 65]. The serum levels of CCL26 were found to be significantly higher in atopic dermatitis patients than in normal individuals or psoriasis patients, and correlated well with the serum levels of CCL17, CCL22, eosinophil numbers and disease severity in atopic dermatitis [64]. Moreover, the CCL26 mRNA and protein levels were enhanced in a human keratinocyte cell line by the addition of IL-4 and strongly enhanced by the combination of either IL-4 and IL-13 or IL-4 and TNF- α , the 3 cytokines known to be upregulated in atopic dermatitis skin lesions, peripheral T cells, or plasma [65, 67, 68]. Besides CC chemokines, the CX3C chemokine CX3CL1 (fractalkine) was also thought to be involved in atopic dermatitis [66]. CX3CL1 protein was strongly expressed in endothelial cells in the skin lesions of atopic dermatitis, but not in normal skin, and its mRNA levels in atopic dermatitis lesional skin increased to a similar extent as those of CCL17 and CCL22 [66]. In addition, serum CX3CL1 levels in atopic dermatitis patients were increased in comparison to normal subjects and correlated with disease severity [66]. Together with the increase in CX3CL1 receptor CX3CR1+ cells in the lesional skin of atopic dermatitis, these data suggest a possible role of CX3CL1 in the pathogenesis of atopic dermatitis [66].

Role of Cutaneous Allergens

Besides the perceived role of cutaneous allergens in the pathogenesis of atopic dermatitis as most patients have *Staphylococcus* colonization in their

skin, there are some experimental evidences that strongly suggest their pathogenic roles. To be inclusive, allergens in this particular context will not be restricted to the typical allergic chemicals, but will also consist of skin pathogens and skin self-proteins. The first indirect evidence that supports the role of cutaneous allergens in the pathogenesis of atopic dermatitis is the fact that some circulating and skin-infiltrating T cells from these patients are allergen-reactive and proliferated strongly in response to skin allergens [25, 28]. The second evidence that bolsters the role of cutaneous allergens in atopic dermatitis is the finding that sufficient amounts of allergen-specific IgE and allergen-reactive T cells occur concomitantly in the blood of patients with atopic dermatitis, allowing IgE-enhanced T cell responses to allergens [41]. Moreover, these allergens could induce skin inflammation when applied to normal-appearing skin of patients with atopic dermatitis [69]. In another study the disease severity correlated well with a change in the accessory gene regulator groups of the skin-colonized *S. aureus* [70]. Lastly, several self-proteins, namely Hom s 1, Hom s 4, keratohyalin granule component LEDGF/DFS70 and manganese superoxide dismutase, have been identified as putative autoantigens for atopic dermatitis as they are recognized by the IgE class autoantibodies obtained from atopic dermatitis patients [71–74].

Role of Microvascular Angiogenesis

Inflammation-mediated angiogenesis has been suggested to play a role in the propagation of skin inflammation [75, 76]. Angiogenesis has long been thought to play a role in psoriasis, another common inflammatory skin disease that is characterized by histological evidence of dermal microvascular alterations [76]. Because the studies on the role of angiogenesis in inflammation are relatively recent undertakings, definitive data on the role of angiogenesis in human patients of atopic dermatitis are currently not available. One study, however, did examine the expression of vascular endothelial growth factor (VEGF), a proangiogenic factor produced by epidermal cells, in the skin of these patients and found that VEGF expression is upregulated in the lesional skin of atopic dermatitis patients, compared to normal-appearing skin of these patients. Furthermore, they found that VEGF-121 is the exclusive isoform from these patients that shows functional ability to induce hyperpermeability of blood vessels [77].

Data from Animal Model Investigations

Essentially all data collected from animal models of atopic dermatitis thus far were obtained from extrinsic models, characterized by high levels of total serum IgE. This will make the interpretation easier. Many animal models, including those conducted in small animals such as mice [78, 79] and those conducted in larger animals such as feline and canine [80, 81], have been utilized to



Fig. 1. Typical clinical phenotype of the K14-IL-4-Tg mouse, manifesting chronic inflammation primarily in the ear, mouth and periorbitally.

study the pathogenesis of atopic dermatitis. By far the most characterized small animal model is the spontaneous Nc/Nga mouse model established by Japanese investigators in the late 1990s [82, 83]. Most of these mouse and canine models have been described in details in 2 recently published textbooks of in vivo animal models of inflammation [84, 85]. In this chapter, I will focus on a transgenic mouse model of extrinsic atopic dermatitis, in which a critical Th2 cytokine, IL-4, has been overexpressed in the basal level of mouse epidermis through an epidermis-specific keratin 14 (K14) promoter/enhancer (fig. 1) [86–91]. Two of the major advantages of this K14-IL-4-Tg mouse model of atopic dermatitis are that the disease induction is 100% in the Tg mice (compared to 0% in non-Tg mice) [87–90] and that as a group these Tg mice developed a chronic, itchy, inflammatory skin disorder closely resembling human atopic dermatitis and actually fulfilled the clinical diagnostic criteria for human atopic dermatitis according to the 1980s criteria established by Hanifin and Rajka (table 1) [6, 86]. Specifically, this K14-IL-4-Tg mouse model fulfilled 3 major criteria of pruritus, chronic dermatitis and family history of atopy, and 4 minor criteria of xerosis, elevation of serum IgE, *Staphylococcus* skin infection and conjunctivitis [6, 86]. Our IL-4-Tg mouse model of atopic dermatitis not only fulfilled the clinical diagnostic criteria for human atopic dermatitis, but also provided experimental data, as can be seen below, consistent with those derived from studies on human atopic dermatitis when available. Interpreting data from animal model studies, nevertheless, requires the understanding that immune mechanisms occurring in animals

may not be applicable in their entirety to humans, since there are some minor differences between the immune systems of animals and humans [92]. For example, FcεRI, the high-affinity IgE receptor that is present in human Langerhans cells, is not found in mice [92–95]. Another example is the fact that while in the mouse humoral immune system, the production of IgG1 is predominantly influenced by cytokines produced by Th2 lymphocytes and the synthesis of IgG2a is primarily induced by Th1 lymphocytes, such clear divisions are not yet established in the human humoral immune system and the nomenclature of human IgG isotypes (IgG1, IgG2, IgG3 and IgG4) is also different from that of mouse [31, 96]. In order to determine the immunological sequence of events occurring in our IL-4-Tg mouse model of atopic dermatitis with step-by-step measurement of immunological parameters at different stages of disease development, we consistently utilized 4 groups of mice in all our experiments: IL-4-Tg mice before disease onset (Tg-BO), Tg mice with acute/early disease stage (Tg-EL, lesion duration less than 1 week), Tg mice with chronic/late disease stage (Tg-LL, lesion duration more than 3 weeks) and non-Tg mice as negative control. In some studies, normal-appearing skin of Tg mice with chronic disease stage (Tg-NL) was also included.

Role of T Cells

Like in human patient studies, the lesional skin of IL-4-Tg mice is characterized by infiltration of CD3+ T cells, both CD4+ and CD8+ subsets [88]. Both subsets of T cells are detected infiltrating into the epidermis and the dermis. CD4+ T cells dominate the dermal infiltrate, whereas CD8+ T cells are larger in number in the epidermis [88]. When the T cells in the lymphoid organs were examined, we found that as the disease progressed from Tg-BO stage to Tg-EL and Tg-LL stages, the percentage of cells expressing the surface-activation molecules CD44 and CD69 in both CD4+ and CD8+ subsets increased in parallel to disease advancement (fig. 2) [88]. Furthermore, the percentage of CD4+ and CD8+ T cells bearing the costimulatory molecules ICOS and PD-1 also similarly increased as the disease progressed [88]. The activation states of the T cells in the Tg mice were further confirmed by proliferation assays performed on inflammatory cells derived from lymphoid organs. The leukocytes, freshly isolated from the lymphoid organ of the mice in Tg-EL and Tg-LL disease stages, spontaneously proliferate in the culture medium without additional stimulant, compared to that of the non-Tg mice. Moreover, when stimulated by antibody to CD3, Con A, phytohemagglutinin or staphylococcal enterotoxins A and B, these lymphoid cells proliferate to significantly greater extent than those of non-Tg mice, indicating that the T cells are highly activated in our model of inflammatory disease (fig. 3) [88].

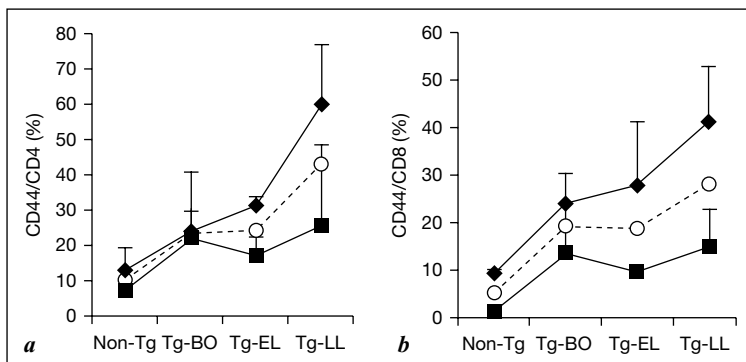


Fig. 2. Flow-cytometric analyses of lymphoid organ leukocytes demonstrating the correlated increase in the percentage of the cell surface activation molecule CD44 among CD4+ (a) and CD8+ (b) T cells in the spleen (solid diamonds) and skin-draining lymph node (solid squares) with disease progression. Hollow circles are the average values between spleen and lymph node. Reprinted with permission from Chen et al. [88].

Role of Antigen-Presenting Cells

So far, our model has provided little evidence to support the role of antigen-presenting cells for the pathogenesis of skin inflammation [88]. By examining the presence of surface activation molecules of all CD11c+ dendritic cells of the lymphoid organ, we found that the total number of dendritic cells, the percentage of dendritic cells (among all CD11a+ bone marrow-derived leukocytes) and the percentage of costimulatory molecule-bearing dendritic cells occurred in the mice in the Tg-BO disease stage. The costimulatory molecules we examined included CD80, CD86, B7h and B7-DC [88]. We also found that another subset of antigen-presenting cells, macrophages, is activated. The total number of Mac3+ cells, as well as the percentage of Mac3+ cells (among CD11a+ bone marrow-derived leukocytes), increased as the disease progresses from Tg-BO to Tg-EL, and then to Tg-LL disease stages (fig. 4) [88]. Similarly, when stimulated by antibody to staphylococcal enterotoxin B, these lymphoid cells, whether they are from the mice in Tg-BO, Tg-EL or Tg-LL disease stages, proliferate to significantly greater extent than those of non-Tg mice (fig. 3). Together, these data suggest that the antigen-presenting cells are highly activated, even before the disease onset, and may play a role in activating T cells, leading to the inflammatory responses in the skin.

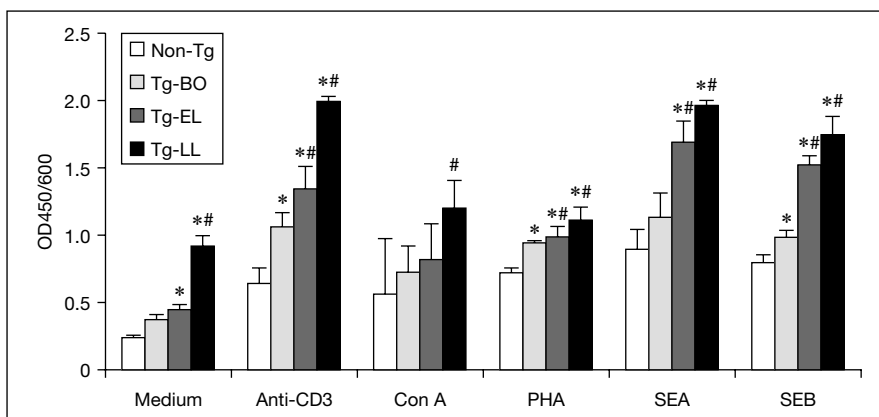


Fig. 3. T cell proliferation assays illustrating the spontaneous proliferative nature of the skin-draining lymph node leukocytes of the K14-IL-4-Tg mice, with enhanced proliferative potential in nonstimulated culture medium and enhanced proliferative nature of these cells when stimulated with T cell-activating antibody (anti-CD3), mitogens (Con A and phytohemagglutinin) and superantigens (*Staphylococcus* enterotoxin A and B). PHA = Phytohemagglutinin; SEA = *Staphylococcus* enterotoxin A; SEB = *Staphylococcus* enterotoxin B. *Statistical significance versus non-Tg mice; #statistical significance versus Tg-BO mice. Reprinted with permission from Chen et al. [88].

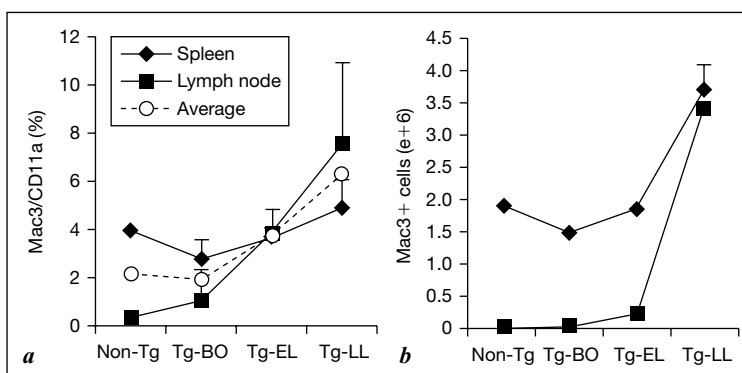


Fig. 4. Flow-cytometric analyses of lymphoid organ leukocytes demonstrating the correlated increase in the percentage of Mac3+ macrophages among CD11a+ bone marrow-derived leukocytes (a) and total numbers of Mac3+ (b) macrophages in the spleen and skin-draining lymph nodes with disease progression. Reprinted with permission from Chen et al. [88].

Table 4. Increase in skin cytokine expression of K14-IL-4-Tg mice by cDNA microarray

Cytokine type	Cytokines	Tg-LL/non-Tg, n-fold increase
Th2	IL-3	9.9 ± 2.5
	IL-4	6.8 ± 2.2
	IL-5	9.7 ± 4.1
	IL-6	11.0 ± 3.0
	IL-10	6.2 ± 2.3
	IL-13	4.1 ± 2.1
Th1	IL-2	3.2 ± 1.0
	IL-12p40	7.2 ± 3.1
	IFN- γ	8.8 ± 1.1
	TNF- β	5.7 ± 2.3
Non-Th	IL-1 β	23.1 ± 2.0
	TNF- α	4.9 ± 1.8

Derived from Chen et al. [87].

Role of Cytokines

In order to characterize the role cytokines play in the initiation and maintenance of skin inflammation in the IL-4-Tg mice, an extensive survey of cytokines present in the skin was conducted, using non-Tg mice as controls, with 4 groups of skin samples from IL-4-Tg mice in the Tg-BO, Tg-EL and Tg-LL disease stages, plus Tg-NL skin. Normal-appearing skin samples (for non-Tg and Tg-BO mice) and inflamed skin samples (for Tg-EL and Tg-LL mice) were collected and stored in RNA-protecting medium (RNAlater). The total RNAs extracted from these tissues were first reverse transcribed to cDNA, followed by examinations using 2 different methods: cDNA microarray and real-time PCR [87]. When screening the cytokine expressions by cDNA microarray, we found that substantial increases in mRNAs in various Th2 cytokines (IL-3, IL-4, IL-5, IL-6, IL-10 and IL-13), Th1 cytokines (IL-2, IL-12, IFN- γ and TNF- β), as well as non-Th proinflammatory cytokines (IL-1 β and TNF- α) occurred in mice samples of Tg-LL stage compared to samples from non-Tg mice (table 4). We then validated our findings in the cDNA microarray by using the quantitative PCR methodology (fig. 5). We confirmed that essentially all cytokines we tested in the mouse samples of Tg-LL stage, including Th2 cytokines (IL-3, IL-4, IL-6, IL-10 and IL-13, except IL-5), 2 Th1 cytokines

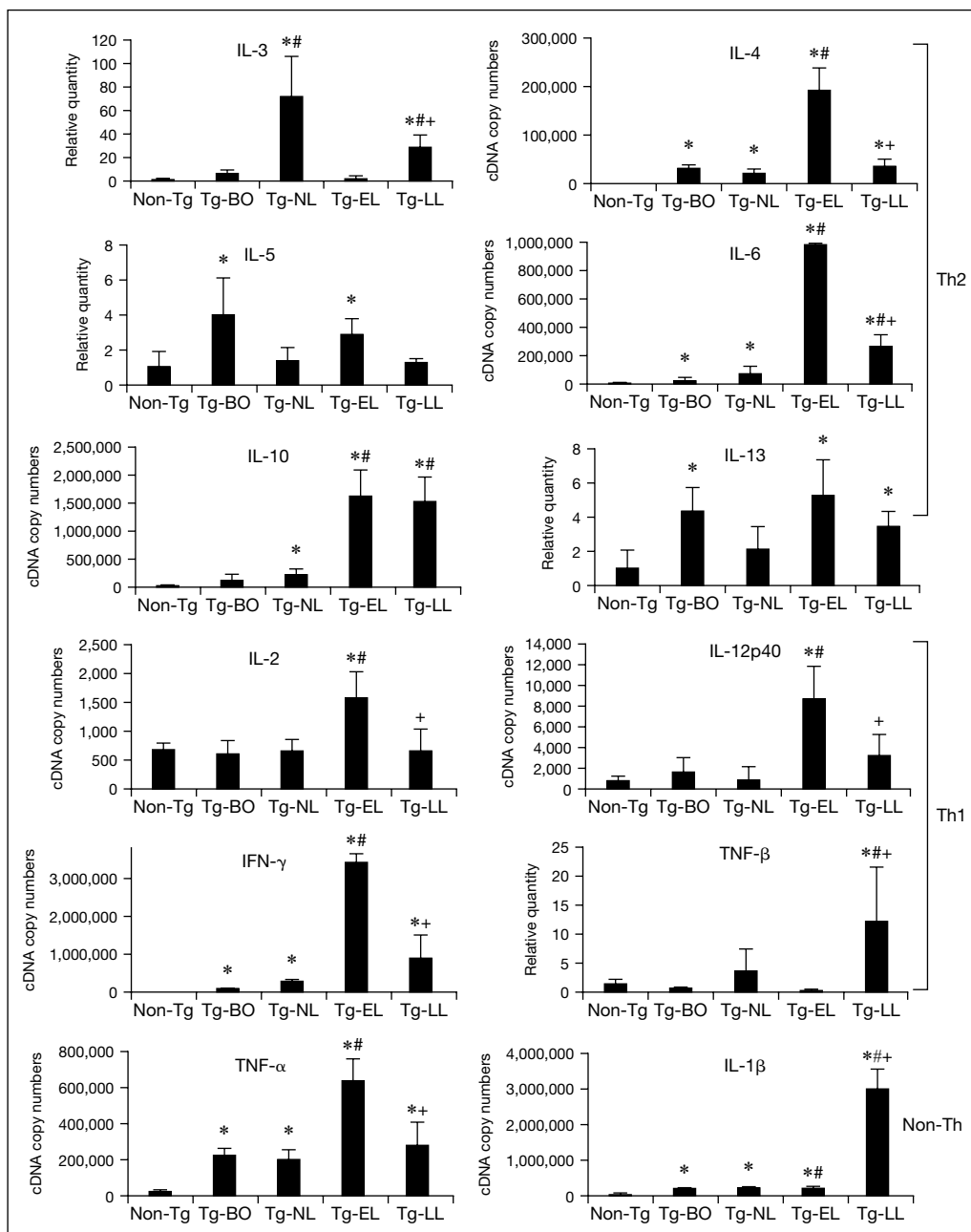


Fig. 5. Quantitative analyses of cytokine mRNA expression levels of the skin using real-time PCR on cDNA reverse transcribed from total RNA showing an early upregulation

(IFN- γ and TNF- β) and non-Th proinflammatory cytokines (IL-1 β and TNF- α) were significantly increased in comparison to the samples from non-Tg mice (fig. 5). Importantly, the Th2 cytokines tend to peak at the earlier disease stages (Tg-NL, Tg-BO or Tg-EL), whereas the Th1 cytokines tend to peak at the later stages (Tg-EL or Tg-LL; fig. 5). Together, these data seem to indicate that there is an early upregulation of Th2 cytokines followed by a late surge of Th1 cytokines, a pattern very similar to that observed in human atopic dermatitis patients [32, 33]. Next, we determined that as the disease progresses from Tg-EL to Tg-LL stages, the percentage of cytokine-producing cells for various cytokines (IL-2, IL-4, IL-10, IFN- γ and IL-12) in the skin lesions increase as well [87]. This finding suggests the presence of these cytokine-producing cells in the skin could be responsible for continuous progression of the inflammatory disease process.

Role of IgE

As an extrinsic atopic dermatitis model, the IL-4-Tg mouse model is characterized by upregulation of total serum IgE [86, 89]. In order to better understand the pathomechanism leading to the hyperproduction of IgE, we performed extensive investigation of the molecular and cellular mechanisms of B cells and their IgE production [89]. Particularly, we looked into whether these B cells in the mice with inflammatory skin lesions are activated and whether the activation states, as assayed by B cell proliferation and flow-cytometric analysis of surface activation molecules, correlate with disease progression, whether the levels of IgE synthesis by these B cells in the mice with skin inflammation, as measured by flow-cytometric analysis of surface IgE, parallel disease progression, and whether total serum IgE, as determined by ELISA, correlates with disease progression [89]. We found that all the answers were positive. As the disease progresses from Tg-BO to Tg-EL stage, and finally to Tg-LL stage, the intensity of IA/IE surface molecules in the CD19+ B cells obtained from lymphoid organs increases in parallel [89]. Similarly, as the disease progresses, the percentage of CD19+ B cells that express the surface activation molecules CD44 and CD69, and the costimulatory molecules CD80 and CD86, increased in parallel [89]. The activation states of B cells of these Tg mice were subsequently confirmed by B cell proliferation assays, showing that these B cells

of Th2-type cytokines with a late surge of Th1-type cytokines, with respect to disease progression. The cytokines expressed in cDNA copies were obtained with standardized plasmids and the cytokines expressed in relative quantity were obtained without standardized plasmids (with the relative value of non-Tg mice set at 1). *Statistical significance versus non-Tg mice; #statistical significance versus Tg-BO mice; +statistical significance between Tg-EL and Tg-LL groups. Reprinted with permission from Chen et al. [87].

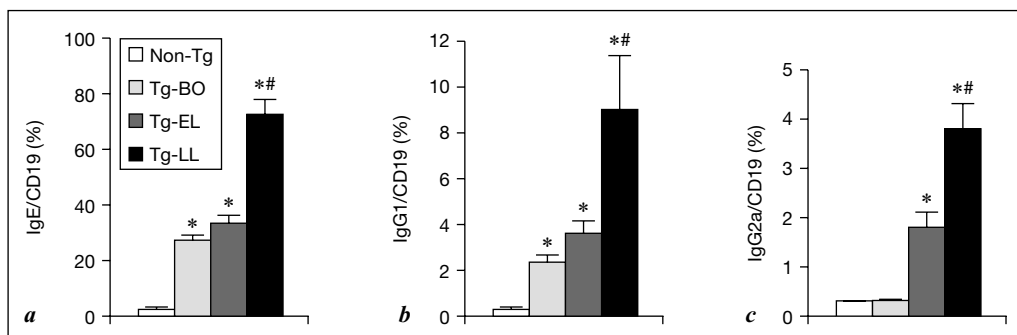


Fig. 6. Flow-cytometric analyses of the skin-draining lymph node CD19⁺ B cells' surface-bound IgE (**a**), IgG1 (**b**) and IgG2a (**c**), illustrating the progressive increase in the percentage of immunoglobulin-bound B cells as the disease evolves. *Statistical significance versus non-Tg mice; #statistical significance versus Tg-BO mice. Reprinted with permission from Chen et al. [89].

increased their proliferative abilities as the disease progressed [89]. Moreover, as the disease progressed, the percentage of cell surface IgG1- and IgE-bearing CD19⁺ B cells increased in parallel (fig. 6). IgG2a-bearing B cells in the cell surface also increased, but only at the Tg-EL and Tg-LL disease stages, consistent with the findings of a late surge of Th1 cytokines documented by our real-time PCR studies of skin cytokines (fig. 5). Finally, the measurements of total serum concentrations of immunoglobulins showed that the trends of increases in IgE, IgG1 and IgG2a are identical to those found in the surface immunoglobulins (figs 6, 7).

Role of Chemokines and Adhesion Molecules

We have sought to identify factors that contributed to the recruitment of T cells into the skin in our IL-4-Tg model and thereby induced inflammation [90]. When we performed real-time PCR experiments on cDNA reverse transcribed from skin-extracted RNAs, we found that many chemokines, including CXCL9, CXCL10, CXCL11, CCL17, CCL22 and CCL27, were upregulated, particularly in the skin samples of mice in the Tg-LL disease stage (fig. 8). The mRNA level of CCL27, a chemokine predominantly produced by epidermal keratinocytes, however, peaked at the Tg-BO stage, suggesting that it plays a role in initiating the disease, since upregulation before disease onset implies its contribution in recruiting inflammatory cells to the skin (fig. 8). We next checked the serum levels of CCL27 by ELISA and indeed found that the protein levels of CCL27 in the mice of Tg-BO are already significantly increased in comparison to those in

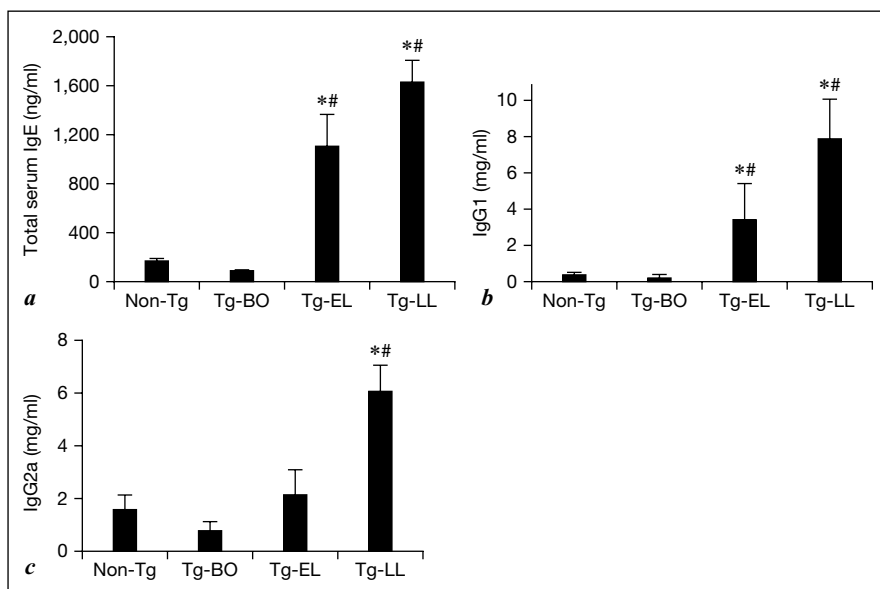


Fig. 7. ELISA assays of total serum concentrations of IgE (**a**), IgG1 (**b**) and IgG2a (**c**), illustrating the progressive increase in total serum immunoglobulin as the disease evolves. *Statistical significance versus non-Tg mice; #statistical significance versus Tg-BO mice. Reprinted with permission from Chen et al. [89].

non-Tg mice (fig. 9). Based on these data, we further investigated the possible role of CCL27 in the pathogenesis of inflammation in our Tg mouse model by examining the presence of CCL27 receptor (CCR10) in the T cells, the T cell migration pattern towards CCL27 in vitro, and in vivo function of CCL27 by antibody blockade [90]. We found that nearly 100% of all CD4+ and CD8+ T cells isolated from the skin lesions of mice in the Tg-EL and Tg-LL stages expressed surface CCR10 (fig. 9). Furthermore, the percentage of lymph node CD4+ T cells that expressed CCR10 progressively increased as the disease progressed (fig. 9). Moreover, the recombinant mouse CCL27, but not an unrelated chemokine CXCL2, specifically enhanced the migration of T cells derived from mice in the Tg-EL and Tg-LL stages in the migration chamber [90]. When we blocked the CCL27 in vivo function by administering monoclonal neutralizing antibody to mouse CCL27 to the Tg mice with early skin lesions, we documented that anti-CCL27, but not isotype control antibody, specifically reduced the disease severity clinically, significantly decreased the epidermal and dermal infiltration inflammatory cells including CD4+ and CD8+ T cells and mast

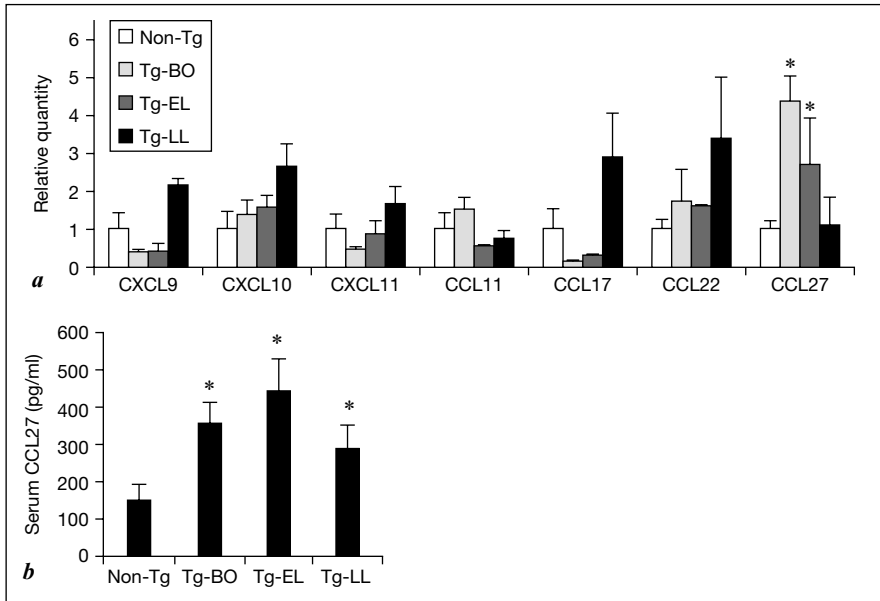


Fig. 8. Quantification of chemokine expressions. **a** Quantitative analyses of skin chemokine mRNA levels using real-time PCR on cDNA reverse transcribed from total RNA, demonstrating upregulation of many chemokine species, particularly in the chronic skin lesions of the K14-IL-4-Tg mice (Tg-LL). **b** ELISA assays of serum chemokine CCL27 showing substantial increase in levels in all disease stage of the Tg mice. $n = 15$. * $p < 0.01$ compared with non-Tg mice. Reprinted with permission from Chen et al. [90].

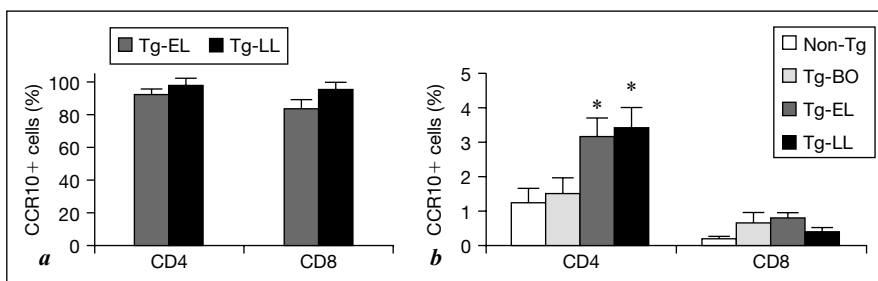


Fig. 9. Quantification of chemokine receptor expressions by flow-cytometric analyses. **a** The majority of CD4+ and CD8+ T cells in the skin lesions expressed CCR10, a receptor for CCL27. **b** Increase in the percentage of CCR10+ T cells in the skin-draining lymph nodes as the disease evolves. * $p < 0.05$ compared with non-Tg mice. Reprinted with permission from Chen et al. [90].

cells histopathologically, and dramatically lowered the expressions of chemokines and cytokines in the skin, including IL-1 β , IL-6, IL-2, IL-12, IFN- γ , TNF- α , macrophage inflammatory peptide-2 (MIP-2), RANTES and CCR10 [90]. In addition, 2 of the non-Th proinflammatory cytokines, IL-1 β and TNF- α , that were significantly upregulated in the skin of mice in the Tg-BO stage by our previous experiments [87], were found to be capable of significantly upregulating CCL27 protein production in primary mouse keratinocytes and the mouse keratinocyte cell line PAM212 [90]. Thus, our data indicated that CCL27 and its T cell receptor CCR10 are very important for T cell recruitment into the skin in this inflammatory disease and that IL-1 β and TNF- α in turn may be responsible for upregulation of CCL27.

Our research on the role of adhesion molecules in the inflammatory response of the IL-4-Tg mice is currently active and ongoing. We have previously reported that by immunofluorescence microscopy performed on skin sections obtained from non-Tg mice and Tg mice in Tg-NL and Tg-LL stages, we found that none of the 4 major endothelial cell adhesion molecules (ICAM-1, VCAM-1, P-selectin and E-selectin) were detectable in non-Tg mice [97]. In contrast, these 4 adhesion molecules were prominently detected in the dermal blood vessels in the skin sections of Tg-LL mice [97]. Furthermore, ICAM-1 was also expressed in the dermal vessels and the epidermis of the Tg-NL skin sections [97]. These preliminary data suggest that these adhesion molecules may play their respective roles in the recruitment of leukocytes into the skin site of inflammation.

Role of Cutaneous Allergens

So far the evidences to support the role of cutaneous allergens collected in the IL-4-Tg mouse model are minimal and indirect [86, 88]. One interesting observation is that the skin lesions developed predominantly in relatively hair-less skin regions, such as the ear (100% involvement; fig. 1), neck (65%), eye (53%), face (29%), legs (12%) and rarely in the heavily haired area of the torso (6%). The finding of this pattern of lesional location suggests that an exposure to allergens is important for disease development, assuming long hair reduces exposure to skin allergens [86]. Moreover, leukocytes derived from skin-draining lymph nodes of mice in the Tg-EL and Tg-LL disease stages proliferated strongly and showed statistically significant increase in response when activated by staphylococcal enterotoxins A and B, in comparison to those of non-Tg mice [88].

Role of Microvascular Angiogenesis

Our investigations of the role of dermal microvascular angiogenesis in the inflammatory response of the IL-4-Tg mice are currently active and ongoing.

These investigations were sparked by the findings of prominent upregulations of microvascular endothelial adhesion molecules present in the skin of mice in the Tg-LL disease stage [97]. When we initiated our studies, no research data were available with regard to the microvascular angiogenesis in human patients of atopic dermatitis or in animal models of atopic dermatitis. Our initial findings of dramatically increased numbers of CD31+ dermal microvasculature in the Tg mice with skin inflammation, relative to non-Tg mice, were rather exciting and also confirmed that there may be a role of microvascular angiogenesis in skin inflammation. We then proceeded to look into the dermal microvasculature at the ultrastructural level and found by transmission electron microscopy that there are substantial evidences in support of the occurrence of angiogenesis in Tg mice with skin inflammation and that the earliest changes occurred in these Tg mice before any clinically observable skin inflammation [91]. In non-Tg mice skin, endothelial cells are stable and quiescent, and arranged regularly on the smooth luminal and subluminal surfaces and thin cell walls. In the skin of mice in the Tg-BO group, early signs of endothelial activation characteristic of angiogenesis were observed: luminal and subluminal protrusions; increased cytoplasmic organelles such as free ribosomes, mitochondria and Golgi apparatus; rough endoplasmic reticulum; increase in the size of the cell, nuclei and nucleoli; basement membrane degradation; sproutings in the capillaries; however, these changes are minor and infrequently observed [91, 98–100]. As the disease progresses from Tg-BO to Tg-EL, and to Tg-LL stage, the characteristic changes of angiogenesis became more prominent and obvious [91]. In the Tg-EL and Tg-LL stages, we frequently found newly formed capillaries identified by their hypertrophied endothelial cells with a cytoplasm rich in organelles and mitochondria and a minuscule lumen. The number and length of interendothelial junctional clefts (IEJC) increased significantly in these new capillaries and the lumens of these capillaries are often occupied by a single erythrocyte [91]. At the same time, the percentage of IEJC that contain tight junctions (TJ) was significantly reduced in these new vessels, strongly suggesting that these vessels are not stable in nature [91]. Similarly, in the postcapillary venules, the average length of junctional complex increased significantly, whereas the average percentage of TJ-containing junctional complexes decreased significantly in the skin of mice in the Tg-EL and Tg-LL stages, compared to non-Tg mice [91].

Two major possible mechanisms of angiogenesis were detected in our Tg mice: sprouting and intussusception. In sprouting, the proliferating endothelial cells sprout and extend into the extravascular matrix and merge into the surrounding vessels with subsequent lumen formed through the connections of the 2 ends of the new and previously existing vessels [101]. On the other hand, the intussusception process involves the continuous introduction of new thin transcapillary pillars, formed by 2 endothelial cell leaflets, resulting in a joint

connection to form junctional complex [101]. By scanning electron microscopic examination of corrosion casts of ear skin obtained from non-Tg mice, we documented that these normal vessels were highly organized and regularly shaped, that there was no redundancy, and that the distance between capillaries was compatible with a low-perfusion bed condition [91]. The smooth cast surface of the blood vessels in the non-Tg mouse skin was in sharp contrast to that of Tg mice revealing the rough and pitted surfaces seen even in the Tg-BO group [91]. The capillary beds of the mice in Tg-EL and Tg-LL stages were chaotically patterned and exuberant, with small vessels connected to other small vessels, apparently haphazardly. In addition, there were substantial amounts of bulbous sprouting, intussusception, protrusion and budding in these diseased vessels, as well as significant degrees of tortuosity [91].

In order to assess the extent of the alterations that occurred in the TJ in the new capillaries of the Tg mice in Tg-EL and Tg-LL stages, confocal microscopy was performed using double labeling of CD31, a blood vessel marker, and claudin-5, a known TJ protein of dermal endothelial cells [102]. We found from our quantitative analysis that the percentage of CD31+ vessels that are claudin-5-positive in the mice in the Tg-EL and Tg-LL stages was significantly reduced compared to that of non-Tg mice and the mice in the Tg-BO stage [91]. The severe reduction of junctional protein correlates well with the finding of dramatic decrease in TJ-containing IEJC documented by transmission electron microscopy [91] and delineates the instability of newly formed vessels on a molecular basis. In addition, our preliminary studies provided evidence that many proangiogenic factors, including VEGF, are upregulated in our IL-4-Tg mouse model, before any angiogenesis study in human patients of atopic dermatitis was reported in the literature [91].

Together, our data collected from transmission and scanning electron microscopic studies as well as confocal microscopic studies provide strong evidence that angiogenesis has indeed occurred in our IL-4-Tg mouse model of atopic dermatitis, that the process of angiogenesis has been initiated before any clinical inflammation is observed, and that angiogenesis has a role in the initiation of the inflammatory process by providing additional and easier paths for the inflammatory cells to reach the inflamed skin sites.

The Skin Barrier Basis of Disease Pathogenesis

Data from Human Patient Studies

The dry appearance (xerosis) of the skin in patients with atopic dermatitis, even in those whose skin lesions are healed or absent, has long been a focal point suggesting the presence of some form of skin barrier defect. Dry skin is in

fact one of the minor diagnostic criteria in the 1980s criteria for atopic dermatitis (table 1) [6]. Many studies have been performed in order to elucidate the actual location and component of the skin involved in such defect and some of these defects are described in the following paragraphs.

Additionally observable phenomena in the clinical situation also support a hypothesis of skin barrier defect in atopic dermatitis. When herpes simplex virus affects these patients, they tend to develop a widespread punched-out lesion, rather than a limited localized lesion observed in most nonatopic individuals [103]. This widespread herpes infection, termed ‘eczema herpeticum’ (or Kaposi’s varicelliform eruption), cannot be explained by a potential immunological defect in atopic dermatitis alone, since the clinical phenotype of eczema herpeticum can also be observed to occur in patients who have other heritable skin diseases due to epidermal defect, termed Darier’s disease and Hailey-Hailey disease (familial benign pemphigus), which are not known to have immunological defect [104–107]. Furthermore, eczema herpeticum has been reported to occur after dermabrasion, a surgical procedure that physically causes skin barrier defect, or in healing burned skin where skin barrier is compromised [108, 109]. Similarly, when atopic dermatitis patients were immunized with smallpox vaccine, the vaccinia virus induced a potentially fatal widespread punched-out lesion termed ‘eczema vaccinatum’ like that of eczema herpeticum, in these patients [110].

In 1998, it was reported that in patients with atopic dermatitis, the levels of the major skin water-holding molecules ceramide 1 and ceramide 3 were significantly lower and the level of cholesterol was significantly higher in the skin lesions, compared to healthy individuals [111]. Furthermore, the levels of ceramide 3 correlated well with transepidermal water loss, suggesting the reduction of skin ceramide content may be involved in barrier dysfunction in atopic dermatitis. Subsequently, it was reported in 1999 that in bacterial skin flora the activity of ceramidase, an enzyme that breaks down ceramide into sphingosine and fatty acid, may be a possible cause of ceramide deficiency in atopic dermatitis [112]. It was found that the bacterial flora obtained from the lesional and nonlesional skin of atopic dermatitis patients, in comparison to normal subjects, secreted significantly more ceramidase [112]. In 2000, it was suggested that the highly expressed level of sphingomyelin deacylase, an enzyme that breaks down ceramide, in the skin of atopic dermatitis patients, in comparison to that of normal individuals, may contribute to the ceramide deficiency in atopic dermatitis, since these enzymes may compete for the common substrates of sphingomyelin or glucosylceramide [113]. In 2002, the ceramide deficiency in atopic dermatitis skin was determined to be due to substantial reduction of de novo synthesis of ceramide 3 and ceramide 4 in the lesional skin of these patients [114]. In addition, the composition of ω -hydroxyceramides in

total protein-bound lipids in normal epidermis (46–53 wt %) was substantially reduced in nonlesional (23–28 wt %) and lesional atopic dermatitis skin (10–25 wt %) [114]. It is now generally accepted that not only lesional skin, but also nonlesional skin, in atopic dermatitis patients suffers some degrees of skin barrier alteration [115].

Since optimal skin barrier function is preserved when the presence of sufficient extracellular lipids forms a competent lamellar bilayer system in the skin, investigators have tested whether it is possible to repair skin barrier abnormality in atopic dermatitis by ceramide-dominant barrier repair lipid, alone or in conjunction with other therapeutic treatments [116, 117]. These investigators indeed showed from their clinical trials that ceramide-dominant barrier repair lipids improved atopic dermatitis conditions, and also led to a reduction of transepidermal water loss and restoration of extracellular lamellar membranes of the stratum corneum as documented by electron microscopy [116, 117].

It is now clear that the barrier defects in the skin of atopic dermatitis patients are not only physical in nature, but are also seen in cellular and biochemical parameters [118–123]. These relative innate immune deficiencies and gene polymorphisms recently reported to be associated with atopic dermatitis included Toll-like receptor 2, nucleotide-binding oligomerization domain 1 (NOD1, a keratinocyte cellular sensor for Gram-negative bacteria product), soluble CD14 (a multifunctional keratinocyte receptor for lipopolysaccharide and activator for nuclear factor κ B), natural antimicrobial peptides (sphingosine, dermcidin) and macrophage inflammatory peptide-3 α (MIP-3 α) [118–123]. These data suggest that downregulation of the sensing and responding mechanisms in the skin of atopic dermatitis patients to bacterial invasion may play a role in the pathogenesis of the disease.

Together, the data from these studies on human patients with atopic dermatitis provide some evidences that an epidermal barrier existed in the skin of these patients and that these defects may in some way contribute to the pathogenesis of atopic dermatitis.

Data from Animal Model Investigations

Since currently there is no animal model of atopic dermatitis derived from a primary defect of the skin barrier, there is no available data in this respect.

The Mutation of Filaggrin in Atopic Dermatitis: A Missing Link between Skin Barrier Defect and Immune Dysregulation?

It has been known to the dermatology community for some time that atopic dermatitis is associated with another skin disorder termed ichthyosis vulgaris,

which manifests in dry fish scales particularly in lower extremities [124, 125]. Although globally the exact extent of this association is not known, one study has suggested that about 8% of atopic dermatitis patients in different regions of the world develop the clinical phenotype of ichthyosis vulgaris [124, 126]. In fact, ichthyosis has been included as a minor diagnostic criteria in the 1980s criteria for atopic dermatitis [6] (table 1) and as an associate feature in a more recent set of criteria for pediatric atopic dermatitis (table 3) [9]. However, when a patient has ichthyosis vulgaris, the chance for him or her to develop atopic dermatitis becomes much higher, as approximately 30–50% of patients affected by ichthyosis vulgaris are also affected by atopic dermatitis [124, 125]. Subsequently, ichthyosis vulgaris was found to be associated with a defect in an epidermal protein filaggrin [127]. As early as 1996, a decreased expression of filaggrin was documented by immunohistochemistry and ELISA examinations in the skin of patients with atopic dermatitis [128]. The immunoreactive filaggrin protein was reduced not only in lesional, but also in nonlesional skin obtained from patients with atopic dermatitis [128].

Filaggrin, a 37-kDa epidermis-produced protein, is one of the major structure proteins of the upper epidermis [129]. They form polypeptide aggregate and join a dense protein-lipid matrix cross-linked by transglutaminases, and eventually become part of the cornified cell envelope of the stratum corneum. Filaggrin, in turn, is proteolytically generated from the 400-kDa polypeptide profilaggrin, which is the major component of the keratohyalin granules of the granular layers of the upper epidermis visible by light microscopy [130, 131]. It is believed that the filaggrin-enriched cornified cell envelope of the stratum corneum functions as an epidermal barrier to prevent transepidermal water loss and to hinder the entrance of allergens, toxic chemicals and infectious microorganisms [130]. Because of the findings of association between atopic dermatitis and ichthyosis, Alain Taieb, a French dermatologist and academician, made the daring hypothesis in 1999 that atopic dermatitis is caused by a primary defect in epidermal barrier dysfunction, when the hypothesis of primary immunological defect in atopic dermatitis was predominating [132]. In his hypothesis, a potential link between barrier defect in ichthyosis vulgaris and immune reactions in atopic dermatitis was proposed [132]. Subsequent to Alain Taieb's hypothesis, more evidences have surfaced in 2001, 2004 and 2005 to further support the possible role of filaggrin defect in the disease development in atopic dermatitis [133–135]. A genome-wide analysis has revealed several linkage peaks for atopic dermatitis including a region of 1q21, an area encompassing the filaggrin gene *FLG* locus [133]. Microarray analysis of lesional atopic dermatitis skin showed a decrease in filaggrin mRNA expression [135].

In 2006, common loss of function variants of one of the major epidermal barrier proteins, filaggrin, has been discovered in atopic dermatitis patients, by

using highly specific PCR-based gene mutation detection methods, and this loss of function is now thought to be a major predisposing factor for atopic dermatitis [136]. Extending the works linking filaggrin mutations (*FLG* R501X and 2282del4) to ichthyosis vulgaris [127], these investigators revealed in their initial study that among those patients who have mild ichthyosis vulgaris and are heterozygous for an *FLG* null allele, 44% developed atopic dermatitis ($n = 29$) and that for those patients with severe ichthyosis vulgaris who are homozygous or compound heterozygous for *FLG* null alleles, 76% were affected by atopic dermatitis ($n = 21$) [136]. Thus, atopic dermatitis in this study population seems to be inherited as a semidominant trait: high penetrance in *FLG*-null homozygous or compound heterozygous individuals and lower penetrance in heterozygous individuals [136]. To verify their initial results, these investigators performed studies in a small cohort of 52 Irish pediatric patients of atopic dermatitis and found that the combined allele frequency for the *FLG* R501X and 2282del4 variants was overrepresented in these atopic patients (0.33), whereas the same combined allele frequency was extremely low in an anonymous unselected Irish control population (0.042, $n = 189$) [136]. In order to replicate this *FLG*/atopic dermatitis association and to gain longitudinal information about the population risk of the *FLG* mutation, these investigators performed genotyping of the 2 *FLG* null alleles in 372 Danish children who had been followed prospectively from pregnancy in a cohort study that specifically recruited Danish mothers with asthma (COPSAC study) [136, 137]. Again, they found that the majority of individuals carrying the *FLG* null allele variants had atopic dermatitis (63%), compared to 40% of the individuals with wild-type *FLG* who were affected by atopic dermatitis. Together, 17.5% of all individuals with atopic dermatitis were determined to be carriers of *FLG* null alleles [136]. To examine whether the *FLG*-atopic dermatitis association is also linked to asthma, one of the disorders that also affects some patients with atopic dermatitis, these investigators performed PCR-based genotyping for both *FLG* R501X and 2282del4 variants in 1,008 Scottish schoolchildren with unknown disease status (the population cohort) and in 604 Scottish schoolchildren and adolescents with physician-diagnosed asthma (the asthmatic cohort) from the Tayside area of Northeast Scotland. They determined that a combined frequency of carriers for both of these mutations in the general population cohort of Scotland was 9.6%, whereas it was 15.7% in the asthmatic cohort. Moreover, this combined frequency was increased to 23% in the asthmatic cohort of patients who also had atopic dermatitis [136]. Furthermore, *FLG* null alleles associate more strongly with the combined atopic dermatitis/asthma phenotype than with asthma alone, as 72% of all children in the asthmatic cohort carrying a *FLG* null allele had developed atopic dermatitis, but only 46% of those in this cohort carrying a wild-type *FLG* were affected

by atopic dermatitis [136]. In addition, the exclusion of *FLG* null individuals still resulted in a substantial association between atopic dermatitis and *FLG* heterozygous carriers (odds ratio = 3.1), but the association between *FLG* variants and asthma occurs only in the asthmatic individuals who also had atopic dermatitis, and was not present in those with asthma in the absence of atopic dermatitis (odds ratio = 0.8) [136]. Thus, these data rule out *FLG* variants as an independent risk factor for asthma development and confirm that they are a pre-disposing factor restricted to the clinical subset of asthma occurring in the context of existing atopic dermatitis disorder.

Looking at the substantial increase in prevalence of childhood atopic dermatitis from 6% in the 1960s to 20% in the 1990s, one must not consider barrier defect as the sole factor for atopic dermatitis development, since it is unthinkable from the genetic perspective that the genetic mutation of filaggrin has increased from 6 to 20% in merely 30 years in this affected population. In addition, approximately 40% of atopic dermatitis patients have wild-type *FLG* alleles [136]. Thus, it is likely that skin barrier defect as well as other major factors such as allergens and immune dysregulation play their respective roles in the development and maintenance of atopic dermatitis. Nevertheless, the role of epidermal barrier defect should now be considered as a very important factor for the initiation and maintenance of atopic dermatitis. Another important task ahead of us will be to search for other forms of epidermal barrier defects in those atopic dermatitis patients carrying wild-type *FLG* alleles. From a disease treatment and prevention point of view, the need to protect and repair defective barrier in patients with atopic dermatitis is more important than ever before.

Intrinsic versus Extrinsic Atopic Dermatitis: Implication on Pathological Mechanisms, Disease Diagnosis and Therapeutic Approaches

The Developing Concept of Intrinsic Atopic Dermatitis

In the past 10 years or so, some experts in the field have proposed that there are actually 2 distinct subsets of atopic dermatitis, primarily based on their serum IgE levels (table 5) [11–21]. A subset of patients who have elevated total serum IgE (>150 kU/l), as described to be one of the minor diagnostic criteria by Hanifin and Rajka [6] in 1980, was proposed to be termed extrinsic or allergic atopic dermatitis, whereas the subset of patients who have normal total serum IgE level (<150 kU/l) was termed intrinsic or nonallergic atopic dermatitis [12]. In addition to total serum IgE, extrinsic patients tend to have positive antigen-specific IgE to common inhalants and food allergens. By contrast, intrinsic patients tend to have negative findings [12]. From the data of most reported studies, it seems

Table 5. Feature comparison of intrinsic and extrinsic atopic dermatitis subsets

	Intrinsic subset	Extrinsic subset	Reference
Lesional morphology	Same as extrinsic		
Age of onset	Later	Earlier	12
Gender	Female predominance	Male predominance	12, 20
Total serum IgE	<150 kU/l	>150 kU/l	12
Antigen-specific IgE to common inhalants/foods	Negative	Positive	12
Skin test to common inhalants or foods	Negative	Positive	12
Peripheral eosinophilia	Mild to moderate	Mild to moderate	12
Dermal eosinophilia	Lower	Higher	17, 18
Eosinophil survival	Same as extrinsic		12
ECP	Same as extrinsic	or higher	12, 19
HLA-DR expression of peripheral blood T cells	Same as extrinsic		12
Peripheral blood IL-5 level	Same as extrinsic	of higher	12, 19
Peripheral blood IL-4 level	Low	High	12
B cell activation	CD23+ B cells low	CD23+ B cells high	12
Epidermal dendritic cells	FcεRI/FcγRII ratio <0.5	ratio >1.5	12, 15
Lesional skin cytokine level	IL-5, IL-13 < extrinsic		12, 17
	IL-1β < extrinsic		17
	IFN-γ, IL-12, IL-4, IL-10, GM-CSF same as extrinsic		17
	TNF-α reduced	TNF-α reduced	17
IL-4 and IL-4R polymorphism	Lower	Higher	16

that the major subset of atopic dermatitis is the extrinsic form, consisting of about 70–80% of all patients, while the intrinsic subset comprises about 20–30% of the patients [20]. Besides the serum IgE levels, extrinsic atopic dermatitis also differs from intrinsic atopic dermatitis in clinical and immunological parameters. Although they do not differ in clinical morphology, extrinsic patients tend to have earlier disease onset and a slightly higher male predominance than intrinsic patients [12, 15, 20]. Immunologically, extrinsic patients tend to have higher peripheral blood IL-4 levels, higher CD23+ B cell levels, higher expression levels of FcεRI/FcγRII ratio in epidermal dendritic cells [15], more prominent eosinophil and eosinophil granular proteins in lesional skin, and higher IL-5 and IL-13 expression levels in lesional skin [12, 17, 18]. Furthermore, lesional skin cytokine levels of IL-1β are higher in the extrinsic subset, whereas the same

levels of IL-2, IL-4, IL-10, GM-CSF and IFN- γ are highly expressed in both subsets [17]. Interestingly, the lesional skin mRNAs of TNF- α were the same in both subsets, but lower than in normal skin [17]. In addition, IL-4 and IL-4 receptor polymorphisms are found more frequently in the extrinsic subset [16]. Some other immunological parameters, such as peripheral eosinophilia, eosinophil survival, eosinophil cationic protein (ECP), HLA-DR expression in peripheral T cells, peripheral blood IL-5 level, dermal CD4/CD8 T cell ratios and epidermal Langerhans cell expressions, showed no difference between these 2 subsets [12, 19]. As it stands, there seems to be some degree of differences between the extrinsic and the intrinsic subsets of atopic dermatitis. Obviously, more work is needed to sort out the underlying basic mechanisms accounting for these differences.

The Diagnostic Implication of the New Intrinsic Atopic Dermatitis

While the concept of the 2 subsets of atopic dermatitis has been proposed by the European investigators [11–16], it has subsequently been supported by some Asian [17–19] and Australian [20] investigators. The US investigators, however, have been largely silent in this aspect, with regard to investigative efforts. A recent European report of a low occurrence of intrinsic atopic dermatitis (~5%) in an adult population has raised the question regarding the true extent and value of this subset and the participation of US investigators in this regard will be very helpful in settling this question [21]. One alarming observation is that some of the patients initially categorized as having intrinsic atopic dermatitis later developed IgE-mediated sensitization, therefore converting into extrinsic atopic dermatitis [21]. Thus, it seems that a more vigorous method to define this intrinsic subset will require the confirmation by multiple tests on serum IgE at various phases of the disease, as well as long-term follow-up of these patients in order to monitor the possible changes of their intrinsic status.

The Patient Management Implication of the New Intrinsic Atopic Dermatitis

Along with the diagnostic challenge in defining the new intrinsic atopic dermatitis subset, the physicians will also face the ultimate challenge of deciding (1) whether there is a difference in the disease pathogenesis between extrinsic and intrinsic atopic dermatitis, and (2) if such a difference exists, whether different approaches in managing the patients of these 2 subsets of atopic dermatitis are warranted. To sum up, the proper disease management will depend on our understanding of the underlying mechanisms accounting for the differences between these 2 subsets. Obviously, if the distinction between these 2 subsets passes the test of long-term scientific scrutiny, treatments directly targeting the overproduction of serum IgE in patients of extrinsic atopic dermatitis would not be useful in the patients affected by the intrinsic subset. In light of

the newly found evidence of filaggrin mutations occurring in some but not all patients of atopic dermatitis, the investigations of whether there are differential genotypic and phenotypic expressions of this epidermal barrier protein and other barrier proteins in these 2 subsets of patients will be a prudent and timely undertaking and be very beneficial to the medical community.

Advances in Disease Management: Present and Future Approaches

Diagnostic Advances

One of the major advances of atopic dermatitis diagnosis is the ‘atopy patch test’, which is available for the identification of allergens in aeroallergen-triggered atopic dermatitis [138].

Therapeutic Advances

Despite the many intensive studies in this area, topical corticosteroid remains the mainstay of treatment for atopic dermatitis. This group of medications still holds its major role in treating atopic dermatitis, in part due to its relatively rapid onset of action and in part due to its relatively low systemic absorption rate and therefore less systemic side effects, particularly when used properly for adult patients. When used in very young patients, its side effects are more problematic in that there is a potential for adrenal suppression [139].

Having stated the central role of topical corticosteroids, we must also recognize the substantial benefits we gained from topical calcineurin inhibitors [140–149]. In the last few years, the medical community has gained much understanding on this group of medications regarding its desirable actions and potential side effects [140–149]. Specifically, these inhibitors improved skin inflammation in patients with atopic dermatitis, likely through the mechanisms of regulatory T cell correction [141], depletion of plasmacytoid dendritic cells [142], suppressing expressions of the cytokine IL-5, the chemokines eotaxin and RANTES, and the chemokine receptor CCR3 [145], induction of apoptosis-mediated T cell depletion [146], reduction of dermal infiltration of cytokine-expressing inflammatory cells [147, 148], and suppression of skin response to aeroallergens [143], while at the same time they do not appear to hinder the immune system to respond to vaccination [140, 149]. The recent ‘black box’ warning label placed by the FDA on these inhibitors casts a cloud for the long-term use of this group of medications. The readers are encouraged to consider the response from the American Academy of Dermatology [144] as well as to consult 2 other most recent publications regarding the guidelines for using these medications and topical corticosteroids [150, 151].

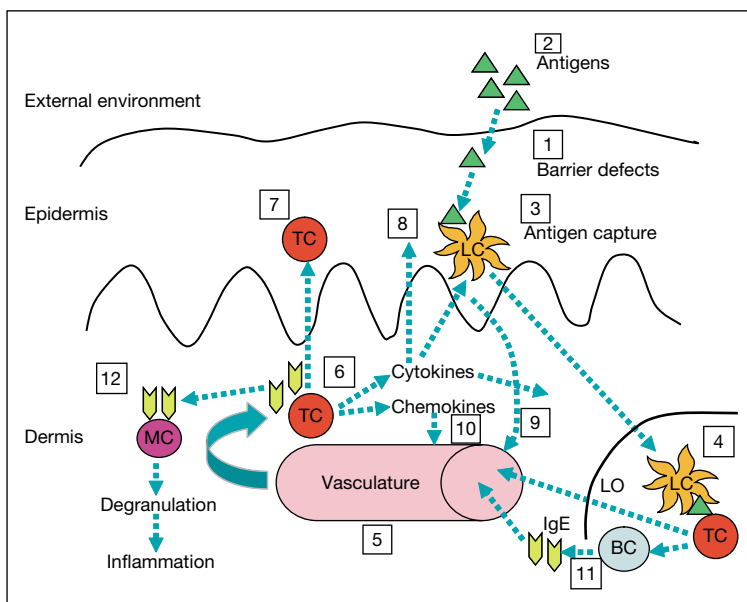


Fig. 10. Schematic illustration of a proposed pathophysiology of extrinsic atopic dermatitis. The barrier defects existing in patients with atopic dermatitis open the door for easy penetration of allergens/pathogens into the epidermis of the skin. The entry of allergens/pathogens likely elicits an immune response from the patients in the following pathways. The allergens/pathogens are captured by epidermal Langerhans cells and/or other epidermal or dermal dendritic cells, which then migrate to the skin-draining lymph nodes or other lymphoid organs to present the processed antigens to the T cells. The T cells activated in the lymphoid organs then migrate towards the skin via the cutaneous microvasculature. Upon arrival in the dermis of the skin, the activated skin-homing T cells secrete various cytokines which affect both the epidermis and dermal vasculature, resulting in upregulation of cytokines and chemokines by the epidermis (for example CCL27, IL-1 β and IL-6) and upregulation of adhesion molecules by dermal vascular endothelial cells (for example ICAM-1, VCAM-1, E-selectin and P-selectin). The upregulation of epidermis-secreted IL-6, along with T cell-produced IFN- γ , then activates keratinocytes to upregulate their production of VEGF, resulting in inflammation-mediated angiogenesis by the actions of VEGF on endothelial cells and blood vessels. The upregulation of CCL27 in the epidermis becomes an enhanced T cell-recruiting force by chemoattracting more CCL27 receptor-bearing (CCR10+) T cells into the dermis, with the assistance of upregulated endothelial adhesion molecules and angiogenic microvasculature. The activated T cells from the lymphoid organs also activate B cells, in the presence of IL-4 and IL-2, causing B cells to become committed IgE-producing plasma cells. The overproduced IgE then travel via the cutaneous microvasculature to the dermis, where they bind and cross-link the surface IgE receptors of the mast cells, leading to mast cell degranulation that further enhances the inflammatory process by the release of mast cell mediators such as histamines and leukotrienes. The dermal T cells also travel into the epidermis, causing further skin structure alterations. The T cell-producing cytokines, in addition, participate in inducing the

Other potentially beneficial therapeutic regimens that are on the horizon include prebiotic oligosaccharides, probiotic *Lactobacillus* strain, synbiotics [152–154], chemokine receptor antagonists [155], anti-IgE monoclonal antibody [156], leflunomide, an immunosuppressant directed against pyrimidine de novo synthesis [157], and medication against SOCS-3, a family of suppressors of cytokine signaling, with functions in directing Th2 responses [158].

Prevention Advances

The findings of epidermal barrier defect in general and the discoveries of *FLG* mutations in particular have had and will continue to have a significant impact on the development of methods and commercial products specifically gearing towards the protection of impaired epidermal barrier and the repair of defective epidermal barrier. As mentioned above in Data from Human Patient Studies, the experimental use of ceramide-containing emollient has been proved to be useful in small studies [116, 117]. This is a very target-specific approach gearing to replace exactly what was missing in the skin of patients affected by atopic dermatitis and hopefully it will become a very useful therapeutic option in the not-too-distant future. Another approach that we are in great need of study will be a replacement method for filaggrin, in light of the recent discovery of *FLG* mutations in atopic dermatitis patients. A direct replacement of filaggrin, whether by a genetic approach, such as in some form of gene therapy, or by a protein approach, such as in some form of topical protein replacement, should be initiated and tested for its usefulness, on both short- and long-term bases, since the skin is so assessable and provides such an outstanding opportunity to test and obtain a solution for the patients we care for on a daily basis.

Conclusion

As we enter 2008, 75 years after Wise and Sulzberger first introduced the concept of atopic dermatitis in 1933 [1, 3], a clearer picture of how atopic dermatitis is initiated, maintained and perpetuated has been emerging, credited to tremendous efforts by the investigators who worked with human patients affected by atopic dermatitis and those who conducted experiments with animal models. Such an updated picture of pathogenesis of the more common extrinsic atopic dermatitis will look something like the diagram in figure 10, while a clearer picture for the pathomechanism of the less common intrinsic atopic der-

hyperproliferation of the epidermal cells, resulting in acanthosis, hyperkeratosis and parakeratosis observed in histopathology. TC = T cells; LC = Langerhans cells; MC = Mast cells; LO = lymphoid organs; BC = B cells.

matitis remains to be further elucidated. Having obtained a fuller understanding of the pathogenesis of the disease, both the clinicians and the scientists could now better target the disease by focusing on 2 major pathways: (1) prevention of the disease by vigorously protecting the impaired skin barrier and repairing the barrier defects, and (2) improvement of the condition when the disease develops by targeting the multiple components identified as potential factors responsible for disease initiation or maintenance, such as T cells, antigen-presenting cells, cytokines, chemokines, adhesion molecules, proangiogenic factors and IgE overproduction.

References

- 1 Wuthrich B: What is atopy? Condition, disease, or a syndrome?; in Wuthrich B (ed): *The Atopy Syndrome in the Third Millennium*. Curr Probl Dermatol. Basel, Karger, 1999, vol 28, pp 1–8.
- 2 Coca AF, Cooke RA: On the classification of the phenomena of hypersensitiveness. *J Immunol* 1923;8:153–182.
- 3 Wise F, Sulzberger MB: Footnote on problem of eczema, neurodermatitis and lichenification; in Weis F, Sulzberger MB (eds): *The 1933 Year Book of Dermatology and Syphilology*. Chicago, Year Book, 1933, pp 38–39.
- 4 Stiffler WC: A 21 year follow-up of infantile eczema. *J Pediatr* 1965;66:166–169.
- 5 Musgrove K, Morgan JK: Infantile eczema: a long-term follow-up study. *Br J Dermatol* 1976; 95:365–372.
- 6 Hanifin JM, Rajka G: Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;92:44–47.
- 7 Williams H: Disease definition and measures of disease frequency. *J Am Acad Dermatol* 2001;45: S33–S36.
- 8 William HC: What is atopic dermatitis and how should it be defined in epidemiological studies?; in Williams HC (ed): *Atopic Dermatitis*. Cambridge, Cambridge University Press, 2000, pp 3–24.
- 9 Eichenfield LF, Hanifin JM, Luger TA, Stevens SR, Pride HB: Consensus conference on pediatric atopic dermatitis. *J Am Acad Dermatol* 2003;49:1088–1095.
- 10 Ring J: Perspectives of atopic eczema in the third millennium; in Wuthrich B (ed): *The Atopy Syndrome in the Third Millennium*. Curr Probl Dermatol. Basel, Karger, 1999, vol 28, pp 192–204.
- 11 Flohr C, Johansson SGO, Wahlgren C-F, Williams H: How atopic is atopic dermatitis? *J Allergy Clin Immunol* 2004;114:150–158.
- 12 Schmid-Grendelmeier P, Simon D, Simon H-U, Akdis CA, Wuthrich B: Epidemiology, clinical features, and immunology of the ‘intrinsic’ (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 2001;56:841–849.
- 13 Novak N, Bieber T: Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 2003;112:252–262.
- 14 Wuthrich B, Schmid-Grendelmeier P: The atopic eczema/dermatitis syndrome: epidemiology, natural course, and immunology of the IgE-associated (‘extrinsic’) and the nonallergic (‘intrinsic’) AEDS. *J Invest Allergol Clin Immunol* 2003;13:1–5.
- 15 Oppel T, Schuller E, Gunther S, Moderer M, Heberstok J, Bieber T, Wollenberg A: Phenotyping of epidermal dendritic cells allows the differentiation between extrinsic and intrinsic forms of atopic dermatitis. *Br J Dermatol* 2000;143:1193–1198.
- 16 Novak N, Kruse S, Kraft S, Geiger E, Kluken H, Fimmers R, Deichmann KA, Bieber T: Dichotomic nature of atopic dermatitis reflected by combined analysis of monocyte immunophenotyping and single nucleotide polymorphisms of the interleukin-4/interleukin-13 receptor gene: the dichotomy of extrinsic and intrinsic atopic dermatitis. *J Invest Dermatol* 2002;119:870–875.

- 17 Jeong CW, Ahm KS, Rho NK, Park YD, Lee DY, Lee JH, Lee ES, Yang JM: Differential in vivo cytokine mRNA expression in lesional skin of intrinsic vs. extrinsic atopic dermatitis patients using semiquantitative RT-PCR. *Clin Exp Allergy* 2003;33:1717–1724.
- 18 Rho NK, Kim WS, Lee DY, Lee JH, Lee ES, Yang JM: Immunophenotyping of inflammatory cells in lesional skin of the extrinsic and intrinsic types of atopic dermatitis. *Br J Dermatol* 2004;151:119–125.
- 19 Park JH, Choi YL, Namkung JH, Kim WS, Lee JH, Park HJ, Lee ES, Yang JM: Characteristics of extrinsic vs. intrinsic atopic dermatitis in infancy: correlations with laboratory variables. *Br J Dermatol* 2006;155:778–783.
- 20 Kusel MM, Holt PG, de Klerk N, Sly PD: Support for 2 variants of eczema. *J Allergy Clin Immunol* 2005;116:1067–1072.
- 21 Folster-Holst R, Pape M, Buss YL, Christophers E, Weichenthal M: Low prevalence of the intrinsic form of atopic dermatitis among adult patients. *Allergy* 2006;61:629–632.
- 22 Zachary CB, Allen MH, MacDonald DM: In situ quantitation of T-lymphocyte subsets and Langerhans cells in the inflammatory infiltrate of atopic eczema. *Br J Dermatol* 1985;112:149–156.
- 23 Sillevs Smitt JH, Bos JD, Hulsebosch HJ, Krieg SR: In situ immunophenotyping of antigen presenting cells and T cell subsets in atopic dermatitis. *Clin Exp Dermatol* 1986;11:159–168.
- 24 Bos JD, Hagenaars C, Das PK, Krieg SR, Voorn WJ, Kapsenberg ML: Predominance of ‘memory’ T cells (CD4+, CDw29+) over ‘naïve’ T cells (CD4+, CD45R+) in both normal and diseased human skin. *Arch Dermatol Res* 1989;281:24–30.
- 25 Santamaria Babi LF, Picker LJ, Perez Soler MT, Drzimalla K, Flohr P, Blaser K, Hauser C: Circulating antigen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen. *J Exp Med* 1995;181:1935–1940.
- 26 Picker LJ, Michie SA, Rott LS, Butcher EC: A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol* 1990;136:1053–1068.
- 27 Akdis M, Akdis CA, Weigl L, Disch R, Blaser K: Skin-homing, CLA+ memory T cells are activated in atopic dermatitis and regulate IgE by an IL-13-dominated cytokine pattern. *J Immunol* 1997;159:4611–4619.
- 28 van der Heijden FL, Wierenga EA, Bos JD, Kapsenberg ML: High frequency of IL-4-producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 1991;97:389–394.
- 29 Lambrecht BN: Allergen uptake and presentation by dendritic cells. *Curr Opin Allergy Clin Immunol* 2001;1:51–59.
- 30 Taylor RS, Baadsgaard O, Hammerberg C, Cooper KD: Hyperstimulatory CD1a+CD1b+CD36+ Langerhans cells are responsible for increased autologous T lymphocyte reactivity to lesional epidermal cells of patients with atopic dermatitis. *J Immunol* 1991;147:3794–3802.
- 31 Kopf M, Gros GL, Bachmann M, Lamers MC, Bluethmann H, Kohler G: Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 1993;362:245–248.
- 32 Grewe M, Fyufko K, Schopf E, Krutmann J: Lesional expression of interferon- γ in atopic eczema. *Lancet* 1994;343:25–26.
- 33 Nakazawa M, Sugi N, Kawaguchi H, Ishii N, Nakajima H, Minami M: Predominance of type 2 cytokine-producing CD4+ and CD8+ cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 1997;99:673–682.
- 34 Chan SC, Li S-H, Hanifin JM: Increased interleukin-4 production by atopic mononuclear leukocytes correlates with increased cyclic adenosine monophosphate-phosphodiesterase activity and is reversible by phosphodiesterase inhibition. *J Invest Dermatol* 1993;100:681–684.
- 35 Tang MLK, Kemp AS: Spontaneous expression of IL-4 mRNA is lymphocytes from children with atopic dermatitis. *Clin Exp Immunol* 1994;97:491–498.
- 36 Ohmen JD, Hanifin JM, Nickoloff BJ, Rea TH, Wyzykowski R, Kim J, Jullien D, McHugh T, Nassif AS, Chan SC, Modlin RL: Overexpression of IL-10 in atopic dermatitis. *J Immunol* 1995;154:1956–1963.
- 37 Marone G, Casolaro V, Cirillo R, Stellato C, Genovese A: Pathophysiology of human basophils and mast cells in allergic disorders. *Clin Immunol Immunopathol* 1989;50:S24–S40.

- 38 Novak N, Kruse S, Potreck J, Maintz L, Jenneck C, Weidinger S, Fimmers R, Bieber T: Single nucleotide polymorphisms of the IL18 gene are associated with atopic dermatitis. *J Allergy Clin Immunol* 2005;115:828–833.
- 39 Sonkoly E, Muller A, Lauerma AI, Pivarsci A, Soto H, Kemeny L, Alenius H, Dieu-Nosjean MC, Meller S, Rieker J, Steinhoff M, Hoffmann TK, Ruzicka T, Zlotnik A, Homey B: IL-31: a new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol* 2006;117:411–417.
- 40 Neis MM, Peters B, Dreuw A, Wenzel J, Bieber T, Mauch C, Krieg T, Stanzel S, Heinrich PC, Merk HF, Bosio A, Baron JM, Hermanns HM: Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis. *J Allergy Clin Immunol* 2006;118:930–937.
- 41 Cooper D, Hales J, Camp R: IgE-dependent activation of T cells by allergen in atopic dermatitis: pathophysiologic relevance. *J Invest Dermatol* 2004;123:1086–1091.
- 42 Fisher PM, Buckley RH: Human IgE biosynthesis in vitro: studies with atopic and normal blood mononuclear cells and subpopulations. *J Immunol* 1979;123:1788–1794.
- 43 Ohman S, Johansson SG: Immunoglobulins in atopic dermatitis with special reference to IgE. *Acta Derm Venereol* 1974;54:193–202.
- 44 Schon MP, Zollner TM, Boehncke WH: The molecular basis of lymphocyte recruitment to the skin: clues for pathogenesis and selective therapies of inflammatory disorders. *J Invest Dermatol* 2003;121:951–962.
- 45 Steinhoff M, Steinhoff A, Homey B, Luger TA, Schneider SW: Role of vasculature in atopic dermatitis. *J Allergy Clin Immunol* 2006;118:190–197.
- 46 Jung K, Linse F, Heller R, Moths C, Goebel R, Neumann C: Adhesion molecules in atopic dermatitis: VCAM-1 and ICAM-1 expression is increased in healthy-appearing skin. *Allergy* 1996;51:452–460.
- 47 de Vries IJ, Langeveld-Wildschut EG, van Reijnsen FC, Dubois GR, van den Hoek JA, Bihari IC, van Wichen D, de Weger RA, Knol EF, Thepen T, Bruijnzeel-Kloomen CA: Adhesion molecule expression on skin endothelia in atopic dermatitis: effects of TNF- α and IL-4. *J Allergy Clin Immunol* 1998;102:461–468.
- 48 Sigurdsson V, de Vries IJ, Toonstra J, Bihari IC, Thepen T, Bruijnzeel-Koomen CA, van Vloten WA: Expression of VCAM-1, ICAM-1, E-selectin, and P-selectin on endothelium in situ in patients with erythroderma, mycosis fungoides and atopic dermatitis. *J Cutan Pathol* 2000;27:436–440.
- 49 Madej A, Reich A, Orda A, Szepletowski JC: Expression of vascular adhesion protein-1 in atopic eczema. *Int Arch Allergy Immunol* 2006;139:114–121.
- 50 Jung K, Imhof BA, Linse R, Wollina U, Neumann C: Adhesion molecules in atopic dermatitis: upregulation of $\alpha 6$ integrin expression in spontaneous lesional skin as well as in atopen, antigen and irritative induced patch test reactions. *Int Arch Allergy Immunol* 1997;13:495–504.
- 51 Pivarsci A, Homey B: Chemokine networks in atopic dermatitis: traffic signals of disease. *Curr Allergy Asthma Rep* 2005;5:284–290.
- 52 Yawalkar N, Uguccioni M, Scharer J, Braunwalder J, Karlen S, Dewald B, Braathen LR, Baggiolini M: Enhanced expression of eotaxin and CCR3 in atopic dermatitis. *J Invest Dermatol* 1999;113:43–48.
- 53 Taha RA, Minshall EM, Leung DY, Boguniewicz M, Luster A, Muro S, Toda M, Hamid QA: Evidence for increased expression of eotaxin and monocyte chemoattractant protein-4 in atopic dermatitis. *J Allergy Clin Immunol* 2000;105:1002–1007.
- 54 Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG: A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA+CCR4+ lymphocytes into lesional atopic dermatitis skin. *J Invest Dermatol* 2000;115:640–646.
- 55 Horikawa T, Nakayama T, Hikita I, Yamada H, Fujisawa R, Bito T, Harada S, Fukunaga A, Chantry D, Gray PW, Morita A, Suzuki R, Tezuka T, Ichihashi M, Yoshie O: IFN- γ -inducible expression of thymus and activation-regulated chemokine/CCL17 and macrophage-derived chemokine/CCL22 in epidermal keratinocytes and their roles in atopic dermatitis. *Int Immunol* 2002;14:767–773.
- 56 Uchida T, Suto H, Ra C, Ogawa H, Kobata T, Okumura K: Preferential expression of T_H2-type chemokine and its receptor in atopic dermatitis. *Int Immunol* 2002;14:1431–1438.
- 57 Shimada Y, Takehara K, Sato S: Both Th2 and Th1 chemokines (TARC/CCL17, MDC/CCL22, and Mig/CXCL9) are elevated in sera from patients with atopic dermatitis. *J Dermatol Sci* 2004;34:201–208.

- 58 Kakinuma T, Saeki H, Tsunemi Y, Fujita H, Asano N, Mitsui H, Tada Y, Wakugawa M, Watanabe T, Torii H, Komine M, Asahina A, Nakamura K, Tamaki K: Increased serum cutaneous T cell-attracting chemokine (CCL27) levels in patients with atopic dermatitis and psoriasis vulgaris. *J Allergy Clin Immunol* 2003;111:592–597.
- 59 Hijnen D, De Bruin-Weller M, Oosting B, Lebre C, De Jong E, Bruijnzeel-Koomen C, Knol E: Serum thymus and activation chemokine (TARC) and cutaneous T cell-attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. *J Allergy Clin Immunol* 2004;113:334–340.
- 60 Hon KL, Leung TF, Ma KC, Li AM, Wong Y, Fok TF: Serum levels of cutaneous T-cell attracting chemokine (CTACK) as a laboratory marker of the severity of atopic dermatitis in children. *Clin Exp Dermatol* 2004;29:293–296.
- 61 Gunther C, Bello-Fernandez C, Kopp T, Kund J, Carballido-Perrig N, Hinterregger S, Fassi S, Schwarzler C, Lametschwandner G, Stingl G, Biedermann T, Carballido JM: CCL18 is expressed in atopic dermatitis and mediates skin homing of human memory T cells. *J Immunol* 2005;174:723–728.
- 62 Nakayama T, Jujisawa R, Yamada H, Horikawa T, Kawasaki H, Hieshima K, Izawa D, Juijie S, Tezuka T, Yoshie O: Induced expression of a CC chemokine liver- and activation-regulated chemokine (LARC)/macrophage inflammatory protein (MIP)-3 α /CCL20 by epidermal keratinocytes and its role in atopic dermatitis. *Int Immunol* 2001;13:95–103.
- 63 Gombert M, Dieu-Nosjean MC, Winterberg F, Bunemann E, Kubitz A, Da Cunha L, Haahtela A, Lehtimäki S, Müller A, Rieker J, Meller S, Pivarski A, Koreck A, Fridman WH, Zentgraf HW, Pavenstadt H, Amara A, Caux C, Kemeny L, Alenius H, Lauerma A, Ruzicka T, Zlotnik A, Homey B: CCL1-CCR8 interactions: an axis mediating the recruitment of T cells and Langerhans-type dendritic cells to sites of atopic skin inflammation. *J Immunol* 2005;174:5082–5091.
- 64 Kagami S, Kakinuma T, Saeki H, Tsunemi Y, Fujita H, Nakamura K, Takekoshi T, Kishimoto M, Mitsui H, Torii H, Komine M, Asahina A, Tamaki K: Significant elevation of serum levels of eotaxin-3/CCL26, but not of eotaxin-2/CCL24, in patients with atopic dermatitis: serum eotaxin-3/CCL26 levels reflect the disease activity and atopic dermatitis. *Clin Exp Immunol* 2003;134:309–311.
- 65 Kagami S, Saeki H, Komine M, Kakinuma T, Tsunemi Y, Nakamura K, Sasaki K, Asahina A, Tamaki K: Interleukin-4 and interleukin-13 enhance CCL26 production in a human keratinocyte cell line, HaCaT cells. *Clin Exp Immunol* 2005;141:459–466.
- 66 Echigo T, Hasegawa M, Shimada Y, Takehara K, Sato S: Expression of fractalkine and its receptor, CX3CR1, in atopic dermatitis: possible contribution to skin inflammation. *J Allergy Clin Immunol* 2004;113:940–948.
- 67 Sumimoto B, Kawai M, Kasajima Y, Hamamoto T: Increased plasma tumor necrosis factor- α concentration in atopic dermatitis. *Arch Dis Child* 1992;67:277–279.
- 68 Antunez C, Torres MJ, Mayorga C, Cornejo-García JA, Santamaria-Babi LF, Blanca M: Different cytokine production and activation marker profiles in circulating cutaneous-lymphocyte-associated antigen T cells from patients with acute and chronic atopic dermatitis. *Clin Exp Allergy* 2004;34:559–566.
- 69 Lomholt H, Andersen KE, Kilian M: *Staphylococcus aureus* clonal dynamics and virulence factors in children with atopic dermatitis. *J Invest Dermatol* 2005;125:977–982.
- 70 Strange P, Skov L, Lisby S, Nielsen PL, Baadgaard O: Staphylococcal enterotoxin B applied on intact normal and intact atopic skin induces dermatitis. *Arch Dermatol* 1996;132:27–33.
- 71 Valenta R, Natter S, Seiberler S, Wichlas S, Maurer D, Hess M, Pavelka M, Grote M, Ferreira F, Szepefalusi Z, Valent P, Stingl G: Molecular characterization of an autoantigen, Hom s 1, identified by serum IgE from atopic dermatitis patients. *J Invest Dermatol* 1998;111:1178–1183.
- 72 Aichberger KJ, Mittermann I, Reininger R, Seiberler S, Swoboda I, Spitzauer S, Kopp T, Stingl G, Sperr WR, Valent P, Repa A, Bohle B, Kraft D, Valenta R: Hom s 4, an IgE-reactive autoantigen belonging to a new subfamily of calcium-binding proteins, can induce Th cell type 1-mediated autoreactivity. *J Immunol* 2005;175:1286–1294.
- 73 Schmid-Grendelmeier P, Fluckiger S, Disch R, Trautmann A, Wuthrich B, Blaser K, Schevnius A, Cramer R: IgE-mediated and T cell-mediated autoimmunity against manganese superoxide dismutase in atopic dermatitis. *J Allergy Clin Immunol* 2005;115:1068–1075.

- 74 Sugiura K, Muro Y, Nishizawa Y, Okamoto M, Shinohara T, Tomita Y, Usukura J: LEDGF/DFS70, a major autoantigen of atopic dermatitis, is a component of keratohyalin granules. *J Invest Dermatol* 2007;127:75–80.
- 75 Detmar M, Brown LF, Schon MP, Elicker BM, Velasco P, Richard L, Fukumura D, Monsky W, Claffey KP, Jain RK: Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice. *J Invest Dermatol* 1998;111:1–6.
- 76 Detmar M, Brown LF, Claffey KP, Yeo KT, Kocher O, Jackman RW, Berse B, Dvorak HF: Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. *J Exp Med* 1994;180:1141–1146.
- 77 Zhang Y, Matsuo H, Morita E: Increased production of vascular endothelial growth factor in the lesions of atopic dermatitis. *Arch Dermatol Res* 2006;297:425–429.
- 78 Gutermuth J, Ollert M, Ring J, Behrendt H, Jakob T: Mouse models of atopic eczema critically evaluated. *Int Arch Allergy Immunol* 2004;135:262–276.
- 79 Zheng T, Zhu Z: Lessons from murine models of atopic dermatitis. *Curr Allergy Asthma Rep* 2005;5:291–297.
- 80 Sinke JD, Thepen T, Bihari IC, Rutten VP, Willemsse T: Immunophenotyping of skin-infiltrating T-cell subsets in dogs with atopic dermatitis. *Vet Immunol Immunopathol* 1997;57:13–23.
- 81 Nuttall TJ, Knight PA, McAleese SM, Lamb JR, Hill PB: Expression of Th1, Th2 and immuno-suppressive cytokine gene transcripts in canine atopic dermatitis. *Clin Exp Allergy* 2002;32:789–795.
- 82 Matsuda H, Watanabe N, Geba GP, Sperl J, Tsudzuki M, Hiroi J, Matsumoto M, Ushio H, Saito S, Askenase PW, Ra C: Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int Immunol* 1997;9:461–466.
- 83 Vestergaard C, Yoneyama H, Murai M, Nakamura K, Tamaki K, Terashima Y, Imai T, Yoshie O, Irimura T, Mizutani H, Matsushima K: Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. *J Clin Invest* 1999;104:1097–1105.
- 84 Chan LS (ed): *Animal Models of Human Inflammatory Skin Diseases*. Boca Raton, CRC Press, 2003.
- 85 Chan LS: Skin inflammatory disorders; in Stevenson CS, Marshall LA, Morgan DW (ed): *In vivo Models of Inflammation*. Basel, Birkhauser, 2006, pp 85–120.
- 86 Chan LS, Robinson N, Xu L: Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. *J Invest Dermatol* 2001;117:977–983.
- 87 Chen L, Martinez O, Overbergh L, Mathieu C, Prabhakar BS, Chan LS: Early up-regulation of Th2 cytokines and late surge of Th1 cytokines in an atopic dermatitis model. *Clin Exp Immunol* 2004;138:375–387.
- 88 Chen L, Martinez O, Venkataramani P, Lin SX, Prabhakar BS, Chan LS: Correlation of disease evolution with progressive inflammatory cell activation and migration in the IL-4 transgenic mouse model of atopic dermatitis. *Clin Exp Immunol* 2005;139:189–201.
- 89 Chen L, Lin SX, Oberbergh L, Mathieu C, Chan LS: The disease progression in the keratin 14 IL-4-transgenic mouse model of atopic dermatitis parallels the up-regulation of B cell activation molecules, proliferation and surface and serum IgE. *Clin Exp Immunol* 2005;142:21–30.
- 90 Chen L, Lin SX, Agha-Majzoub R, Overbergh L, Mathieu C, Chan LS: CCL27 is a critical factor for the development of atopic dermatitis in the keratin-14 IL-4 transgenic mouse model. *Int Immunol* 2006;18:1233–1242.
- 91 Agha-Majzoub R, Becker RP, Schraufnagel DE, Chan LS: Angiogenesis: the major abnormality of the keratin-14 IL-4 transgenic mouse model of atopic dermatitis. *Microcirculation* 2005;12:455–476.
- 92 Chan LS, Gordon KB: Mouse immune system; in Chan LS (ed): *Animal Models of Human Inflammatory Skin Diseases*. Boca Raton, CRC Press, 2003, pp 119–140.
- 93 Bruynzeel-Koomen C, van der Donk EM, Bruynzeel PL, Capron M, de Gast GC, Mudde GC: Associated expression of CD1 antigen and Fc receptor for IgE on epidermal Langerhans cells from patients with atopic dermatitis. *Clin Exp Immunol* 1988;74:137–142.
- 94 Bieber T, de la Salle H, Wollenberg A, Hakimi J, Chizzonite R, Ring J, Hanau D, de la Salle C: Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc ϵ RI). *J Exp Med* 1992;175:1285–1290.

- 95 Hayashi S, Matsuda H, Okumura K, Ra C: Mouse Langerhans cells do not express the high-affinity receptor for IgE. *Arch Dermatol Res* 1999;291:241–243.
- 96 Coffman RL, Savelkoul HF, Lebman DA: Cytokine regulation of immunoglobulin isotype switching and expression. *Semin Immunol* 1989;1:55–63.
- 97 Venkataramani P, Grzeszkiewicz T, Lin S, Prabhakar B, Chan LS: An animal model of atopic dermatitis: lesional skin immunophenotyping delineates a CD4+ T cell-predominated inflammatory infiltrate associated with upregulation of adhesion molecules. *J Invest Dermatol* 2003;121:44.
- 98 Frontezak-Baniewicz M, Walski M: Non-sprouting angiogenesis in neurohypophysis after traumatic injury of the cerebral cortex: electron microscopic studies. *Neuroendocrinol Lett* 2002;23:396–404.
- 99 Masuda H, Kawamura K, Nanjo H, Sho E, Komatsu M, Sugiyama T, Sugita A, Asari Y, Kobayashi M, Ebina T, Hoshi N, Singh TM, Xu C, Zarins CK: Ultrastructure of endothelial cells under flow alteration. *Microsc Res Tech* 2003;60:2–12.
- 100 Schraufnagel DE, Arzouman DA, Sekosan M, Ho YK: The effect of transforming growth factor- α on airway angiogenesis. *J Thorac Cardiovasc Surg* 1992;104:1582–1588.
- 101 Burri PH, Djonov V: Intussusceptive angiogenesis – the alternative to capillary sprouting. *Mol Aspects Med* 2002;23:S1–S27.
- 102 Morita K, Sasaki H, Furuse K, Furuse M, Tsukita S, Miyachi Y: Expression of claudin-5 in dermal vascular endothelia. *Exp Dermatol* 2003;12:289–295.
- 103 Wollenberg A, Wetzel S, Burgdorf WH, Haas J: Viral infections in atopic dermatitis: pathogenic aspects and clinical management. *J Allergy Clin Immunol* 2003;112:6678–6674.
- 104 Parslew R, Verbov JL: Kaposi's varicelliform eruption due to herpes simplex in Darier's disease. *Clin Exp Dermatol* 1994;19:428–429.
- 105 Flint ID, Spencer DM, Wilkin JK: Eczema herpeticum in association with familial benign chronic pemphigus. *J Am Acad Dermatol* 1993;28:257–259.
- 106 Pantazi V, Potouridou I, Katsarou A, Papadogiorgaki TH, Katsambas A: Darier's disease complicated by Kaposi's varicelliform eruption due to herpes simplex virus. *J Eur Acad Dermatol* 2000;14:209–211.
- 107 Donnelly AA, Butler R, Miller CH: A case of Kaposi varicelliform eruption in Darier-White disease. *Cutis* 2005;75:33–36.
- 108 Bestue M, Cordero A: Kaposi's varicelliform eruption in a patient with healing peribuccal dermabrasion. *Dermatol Surg* 2000;26:939–940.
- 109 Bartralot R, Garcia-Patos V, Rodriguez-Cano L, Castells A: Kaposi's varicelliform eruption in a patient with healing second degree burns. *Clin Exp Dermatol* 1996;21:127–130.
- 110 Lane JM, Goldstein J: Adverse events occurring after smallpox vaccination. *Semin Pediatr Infect Dis* 2003;14:189–195.
- 111 Di Nardo A, Wertz P, Giannetti A, Seidenari S: Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. *Acta Derm Venereol* 1998;78:27–30.
- 112 Ohnishi Y, Okino N, Ito M, Imayama S: Ceramidase activity in bacterial skin flora as a possible cause ceramide deficiency in atopic dermatitis. *Clin Diagn Lab Immunol* 1999;6:101–104.
- 113 Hara J, Higuchi K, Okamoto R, Kawashima M, Imokawa G: High-expression of sphingomyelin deacylase is an important determinant of ceramide deficiency leading to barrier disruption in atopic dermatitis. *J Invest Dermatol* 2000;115:406–413.
- 114 Macheleidt O, Kaiser HW, Sandhoff K: Deficiency of epidermal protein-bound ω -hydroxyceramides in atopic dermatitis. *J Invest Dermatol* 2002;119:166–173.
- 115 Jakasa I, Verberk MM, Esposito M, Bos JD, Kezic S: Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. *J Invest Dermatol* 2007;127:129–134.
- 116 Chamlin SL, Frieden HJ, Fowler AJ, Williams M, Kao J, Sheu M, Elias PM: Ceramide-dominant, barrier-repair lipids improve childhood atopic dermatitis. *Arch Dermatol* 2001;137:1110–1112.
- 117 Chamlin SL, Kao J, Frieden HJ, Sheu MY, Fowler AJ, Fluhr JW, Williams ML, Elias PM: Ceramide-dominant barrier repair lipids alleviate childhood atopic dermatitis: changes in barrier function provide a sensitive indicator of disease activity. *J Am Acad Dermatol* 2002;47:198–208.
- 118 Arikawa J, Ishibashi M, Kawashima M, Takagi Y, Ichidawa Y, Imokawa G: Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by *Staphylococcus aureus*. *J Invest Dermatol* 2002;119:433–439.

- 119 Jones CA, Holloway JA, Popplewell EH, Diaper ND, Holloway JW, Vance GH, Warner JA, Warner JO: Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. *J Allergy Clin Immunol* 2002;109:858–866.
- 120 Ahmad-Nejad P, Mrabet-Dahbi S, Breuer K, Klotz M, Werfel T, Herz U, Heeg K, Neumaier M, Renz H: The toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype. *J Allergy Clin Immunol* 2004;113:565–567.
- 121 Weidinger S, Klopp N, Aummier L, Wagenpfeil S, Novak N, Baurecht HJ, Groer W, Darsow U, Heinrich J, Gauger A, Schafer T, Jakob T, Behrendt H, Wichmann HE, Ring J, Illig T: Association of NOD1 polymorphisms with atopic eczema and related phenotype. *J Allergy Clin Immunol* 2005;116:177–184.
- 122 Rieg S, Steffen H, Seeber S, Humeny A, KKalbacher H, Dietz K, Garbe C, Schittek B: Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. *J Immunol* 2005;174:8003–8010.
- 123 Kim BE, Leung DY, Streib JE, Boguniewicz M, Hamid QA, Howell MD: Macrophage inflammatory protein 3 α deficiency in atopic dermatitis skin and role in innate immune response to vaccinia virus. *J Allergy Clin Immunol* 2007;119:457–463.
- 124 Wells RS, Kerr CS: Clinical features of autosomal dominant and sex-linked ichthyosis in an English population. *Br Med J* 1966;1:947–950.
- 125 Kuokkanen K: Ichthyosis vulgaris: a clinical and histopathological study of patients and their close relatives in the autosomal dominant and sex-linked forms of the disease. *Acta Derm Venereol Suppl (Stockh)* 1969;62:1–72.
- 126 Tay YK, Khoo BP, Goh CL: The epidemiology of atopic dermatitis at a tertiary referral skin center in Singapore. *Asian Pac J Allergy Immunol* 1999;17:137–141.
- 127 Smith FJD, Irvine AD, Terron-Kaiatkowski A, Sandilands A, Campbell LE, Zhao Y, Liao H, Evans AT, Goudie DR, Lewis-Jones S, Arseculeratne G, Munro CS, Sergeant A, O'Regan G, Bale SJ, Compton JG, DiGiovanna JJ, Presland RB, Fleckman P, McLean WHI: Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006;38:337–342.
- 128 Seguchi T, Cui CY, Kusuda S, Takahashi M, Aisu K, Tezuka T: Decreased expression of filaggrin in atopic skin. *Arch Dermatol Res* 1996;288:442–446.
- 129 Gan SQ, McBride OW, Idler WW, Markova N, Steinert PM: Organization, structure, and polymorphisms of the human profilaggrin gene. *Biochemistry* 1990;29:9432–9440.
- 130 Candi E, Schmidt R, Melino G: The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 2005;6:328–340.
- 131 Listwan P, Rothnagel JA: Keratin bundling proteins. *Methods Cell Biol* 2004;78:817–827.
- 132 Taieb A: Hypothesis: from epidermal barrier dysfunction to atopic dermatitis. *Contact Dermatitis* 1999;41:177–180.
- 133 Cookson WO, Ubhi B, Lawrence R, Abecasis GR, Walley AJ, Cox HE, Coleman R, Leaves NI, Trembath RC, Moffatt MF, Harper JI: Genetic linkage of childhood atopic dermatitis in psoriasis susceptibility loci. *Nat Genet* 2001;27:372–373.
- 134 Jensen JM, Folster-Holst R, Baranowsky A, Schunck M, Winoto-Morbach S, Neumann C, Schutze S, Proksch E: Impaired sphingomyelinase activity and epidermal differentiation in atopic dermatitis. *J Invest Dermatol* 2004;122:1423–1431.
- 135 Sugiura H, Ebise H, Tazawa T, Tanaka K, Sugiura Y, Uehara M, Kikuchi K, Kimura T: Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope. *Br J Dermatol* 2005;152:146–149.
- 136 Palmer CAN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, Goudie DR, Sandilands A, Campbell LE, Smith FJD, O'Regan GM, Watson RM, Cecil JE, Bale SJ, Compton JG, DiGiovanna JJ, Fleckman P, Lewis-Jones S, Arseculeratne G, Sergeant A, Munro CS, El Houate B, McElreavey K, Halkjaer LB, Bisgaard H, Mukhopadhyay S, McLean WHI: Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441–446.
- 137 Bisgaard H: The Copenhagen prospective study on asthma in childhood (COPSAC): design, rationale, and baseline data from a longitudinal birth cohort study. *Ann Allergy Asthma Immunol* 2004;93:381–389.

- 138 Mohrenschlager M, Darsow U, Schnopp C, Ring J: Atopic dermatitis: what's new? *J Eur Acad Dermatol Venereol* 2006;20:503–511.
- 139 Schlessinger J, Miller B, Gilbert RD, Plott RT: An open-label adrenal suppression study of 0.1% fluocinonide cream in pediatric patients with atopic dermatitis. *Arch Dermatol* 2006;142:1568–1572.
- 140 Hofman T, Cranswick N, Kuna P, Boznanski A, Latos T, Gold M, Murrell DF, Gebauer K, Bechre U, Machura E, Olafsson J, Szalai Z: Tacrolimus ointment does not affect the immediate response to vaccination, the generation of immune memory, or humoral and cell-mediated immunity in children. *Arch Dis Child* 2006;91:905–910.
- 141 Caproni M, Torchia D, Antiga E, Volpi W, del Bianco E, Fabbri P: The effects of tacrolimus ointment on regulatory T lymphocytes in atopic dermatitis. *J Clin Immunol* 2006;26:370–375.
- 142 Hoetzenecker W, Meindl S, Stuetz A, Singl G, Elbe-Burger A: Both pimecrolimus and corticosteroids deplete plasmacytoid dendritic cells in patients with atopic dermatitis. *J Invest Dermatol* 2006;126:2141–2144.
- 143 Weissenbacher S, Traidl-Hoffmann C, Eyerich K, Katzer K, Braeutigam M, Loeffler H, Hofmann H, Behrendt H, Ring J, Darsow U: Modulation of atopy patch test and skin prick test by pretreatment with 1% pimecrolimus cream. *Int Arch Allergy Immunol* 2006;140:239–244.
- 144 Berger TG, Duvic M, van Voorhees AS, Van Beek MJ, Frieden IJ: The use of topical calcineurin inhibitors in dermatology: safety concerns. Report of the American Academy of Dermatology Association Task Force. *J Am Acad Dermatol* 2006;54:818–823.
- 145 Park CW, Lee BH, Han HJ, Lee CH, Ahn HK: Tacrolimus decreases the expression of eotaxin, CCR3, RANTES and interleukin-5 in atopic dermatitis. *Br J Dermatol* 2005;152:1173–1181.
- 146 Hoetzenecker W, Ecker R, Kopp T, Stuetz A, Singl G, Elbe-Burger A: Pimecrolimus leads to an apoptosis-induced depletion of T cells but not Langerhans cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 2005;115:1276–1283.
- 147 Simon D, Vassina E, Yousefi S, Braathen LR, Simon HU: Inflammatory cell numbers and cytokine expression in atopic dermatitis after topical pimecrolimus treatment. *Allergy* 2005;60:944–951.
- 148 Simon D, Vassina E, Yousefi S, Kozlowski E, Braathen LR, Simon HU: Reduced dermal infiltration of cytokine-expressing inflammatory cells in atopic dermatitis after short-term topical tacrolimus treatment. *J Allergy Clin Immunol* 2004;114:887–895.
- 149 Stiehm ER, Roberts RL, Kaplan MS, Corren J, Jaracz E, Rico MJ: Pneumococcal seroconversion after vaccination for children with atopic dermatitis treated with tacrolimus ointment. *J Am Acad Dermatol* 2005;53:S206–S213.
- 150 Hanifin JM, Cooper KD, Ho VC, Kang S, Krafchik BR, Margolis DJ, Schachner LA, Sidbury R, Whitmore SE, Sieck CK, van Voorhees AS: Guideline of care for atopic dermatitis. *J Am Acad Dermatol* 2004;50:391–404.
- 151 Akdis CA, Akdis M, Bieber T, Bindslev-Jensen C, Boguniewicz M, Eigenmann P, Hamid Q, Kapp A, Leung DY, Lipozencic J, Luger TA, Muraro A, Novak N, Platts-Mills TA, Rosenwasser L, Scheynius A, Simons FE, Spergel J, Turjanmaa K, Wahn U, Weidinger JS, Werfel T, Zuberbier T; European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology: Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. *J Allergy Clin Immunol* 2006;118:152–169.
- 152 Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G: A mixture of prebiotics oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 2006;91:814–819.
- 153 Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, Paerregaard A: Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol* 2003;111:389–395.
- 154 Passeron T, Lacour JP, Fontas E, Ortonne JP: Prebiotics and synbiotics: two promising approaches for the treatment of atopic dermatitis in children above 2 years. *Allergy* 2006;61:431–437.
- 155 Elsner J, Escher SE, Forssmann U: Chemokine receptor antagonists: a novel therapeutic approach in allergic diseases. *Allergy* 2004;59:1243–1258.

- 156 D'Amato G, Liccardi G, Noschese P, Salzillo A, D'Amato M, Cazzola M: Anti-IgE monoclonal antibody (omalizumab) in the treatment of atopic asthma and allergic respiratory diseases. *Curr Drug Targets Inflamm Allergy* 2004;3:227–229.
- 157 Schmitt J, Wozel G, Pfeiffer C: Leflunomide as a novel treatment option in severe atopic dermatitis. *Br J Dermatol* 2004;150:1182–1185.
- 158 Seki Y, Inoue H, Nagata N, Hayashi K, Fukuyama S, Matsumoto K, Komine O, Hamano S, Himeno K, Inagaki-Ohara K, Cacalao N, O'Garra A, Oshida T, Saito H, Johnston JA, Yoshimura A, Kubo M: SOCS-3 regulates onset and maintenance of TH2-mediated allergic responses. *Nat Med* 2003;9:1047–1054.

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Cutaneous Lupus Erythematosus: Molecular and Cellular Basis of Clinical Findings

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Abstract

Cutaneous lupus erythematosus (CLE) is a heterogeneous autoimmune disease with well-defined skin lesions, including acute CLE, subacute CLE, chronic CLE and intermittent CLE. In the first part of the review, we discuss the variable relationships that exist between the different clinical forms of CLE and the risk of systemic disease activity. Furthermore, we focus on the annular and papulosquamous forms of subacute CLE and emphasize dermal scarring as a characteristic feature of chronic discoid skin disease in contrast to other subtypes of CLE. Various environmental factors influence the clinical expression of CLE and a striking relationship has emerged between sunlight exposure and the various subtypes of this disease. In the second part, we review the evidence for the abnormal long-lasting photoreactivity in CLE, with an overview of the molecular and cellular factors that may underlie this abnormality. In particular, we discuss the role of UV-mediated induction of apoptosis, mediators of inflammation, such as cytokines and chemokines, nitric oxide, T cell-mediated injury, and the influence of regulatory CD4+CD25+ T cells. However, a complete understanding of the diverse pathophysiologic mechanisms and interactions in CLE does not exist and, as there is yet no convincing animal model of CLE, many studies remain descriptive in nature.

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Clinical Aspects

Variable Relationships Exist between the Different Clinical Forms of Cutaneous Lupus Erythematosus Skin Disease and the Risk of Systemic Lupus Erythematosus Disease Activity

Skin manifestations in lupus erythematosus (LE) are extremely heterogeneous clinically and therefore, it has been difficult to develop a unifying concept of

the various cutaneous lesions of this disease [1]. In 1977, James N. Gilliam initially proposed a classification system [2] and divided the skin manifestations of this disease into those that are histologically specific for LE (LE-specific skin disease) and those that are not histologically specific for this disease (LE-nonspecific skin disease). The LE-specific skin manifestations were primarily based on clinical morphology and included the classic subtypes of cutaneous LE (CLE), such as acute CLE (ACLE), subacute CLE (SCLE) and chronic CLE (CCLE). The adjectives acute, subacute and chronic used in these designations conform to the dermatologic definitions of these terms. However, this classification system does not rigidly define subtypes of CLE, since there are certain patterns of systemic disease activity that can also be seen in conjunction with these variants. In addition, overlapping features can occur with any arbitrary subdivision of a disease as heterogeneous as CLE [3].

Since the initial definition of the nomenclature system by James N. Gilliam, several attempts have been made to improve upon this system and to provide new approaches for the classification of the cutaneous manifestations of this disease [1]. Consequently, this has led to the practice of identifying further subsets, which have been defined by constellations of clinical features, laboratory abnormalities, histologic findings and immunogenetic patterns. Recent analysis of patients with LE tumidus (LET) showed that this subtype has specific characteristic features and that it should be considered as a separate entity [4]. The prognosis in patients with LET is generally more favorable than in patients with other forms of CLE and therefore, a modified classification system, including LET as the intermittent subtype of CLE (ICLE), has been suggested in 2004 (table 1) [5]. Furthermore, there are a number of distinctive forms of LE-nonspecific skin manifestations that will not be addressed in this review, but are included in table 2 for completeness [6]. Examples are cutaneous vasculitis presenting as palpable purpura or urticarial vasculitic lesions, livedo reticularis, rheumatoid nodules and calcinosis cutis. Interestingly, LE-nonspecific skin lesions are often seen in the context of active systemic LE (SLE); however, the molecular basis for the variable relationships that exist between the different clinical forms of CLE skin disease and the risk of SLE disease activity is not yet well understood.

The prognosis of CLE depends on the severity and extent of visceral involvement; however, it is still difficult to predict which cases will develop systemic organ involvement and transition to SLE. Some signs of systemic manifestations, such as minimal proteinuria and arthralgia, are often registered in CLE and 14–27% of patients with discoid LE (DLE) and 67–70% of patients with SCLE have been shown to have mild extracutaneous involvement [7–9]. Several risk factors may influence the course and prognosis of CLE: genetic predisposition, race and sex, clinical presentation, age at disease onset, and

Table 1. Duesseldorf classification of CLE (modified after Kuhn and Ruzicka [5])

-
- ACLE
 - Localized ('malar rash', 'butterfly rash') ACLE
 - Generalized ACLE
 - SCLE
 - Annular SCLE
 - Papulosquamous/psoriasiform SCLE
 - CCLE
 - Discoid LE (DLE)
 - Localized DLE
 - Disseminated DLE
 - Hypertrophic/verruccous DLE
 - LE profundus/LE panniculitis
 - Chilblain LE
 - ICLE
 - LET
-

triggering factors such as exposure to ultraviolet (UV) light and oral medications. Interestingly, SLE patients with associated complement C2 and C4 deficiencies are said to have a better prognosis than individuals without inherited complement deficiencies [10–12]. It seems likely that sex differences also play a role in the course and prognosis of this disease. In one study, 28 males and 111 females with SLE were examined [13]. Disseminated DLE (male 50%/female 21%) and papular and nodular mucinosis (male 18%/female 0%) occurred significantly more commonly in men than in women with SLE and serum sex hormone levels were within the reference range in male patients with this disease. However, other groups reported that men with SLE showed hyperestrogenemia and hypotestosteronemia and suggested that estrogens may facilitate the development of SLE and that testosterone may have protective properties [14, 15]. Follow-up of these patients for a mean of 64 months showed that discoid skin lesions and deeply localized subcutaneous skin lesions occur more frequently in men than in women [16]. However, in another series of patients with SLE including 61 males, no significant difference in the cutaneous manifestations was found between the 2 groups [17].

Further statistical analysis comparing a wide panel of clinical and serologic data in patients with either SLE (n = 464) or CLE (n = 67), mainly DLE, showed that the following parameters were significantly more frequent in SLE than in CLE: female predominance, fever, arthralgia/arthritis, pericarditis, hypertension, pleurisy, oral ulcers, Raynaud's phenomenon, severe headaches, nephritis, proteinuria, anemia, leukopenia, positive antinuclear antibody titer,

Table 2. Nonspecific skin manifestations of LE (modified after Gilliam [2])

-
- Cutaneous vascular disease
 - Vasculitis
 - Leukocytoclastic vasculitis
 - Palpable purpura
 - Urticarial vasculitis
 - Vasculopathy
 - Degos' disease-like lesions
 - Secondary atrophie blanche
 - Periungual telangiectasia
 - Livedo reticularis
 - Thrombophlebitis
 - Raynaud's phenomenon
 - Erythromelalgia
 - Nonscarring alopecia
 - 'Lupus hair'
 - Telogen effluvium
 - Alopecia areata
 - Sclerodactyly
 - Rheumatoid nodules
 - Calcinosis cutis
 - Papulonodular mucinosis
 - Cutis laxa/anetoderma
 - Acanthosis nigricans
 - Erythema multiforme
 - LE-nonspecific bullous lesions
 - Lichen planus
-

high anti-DNA antibody level, low complement C3, and elevated erythrocyte sedimentation rate [18]. In addition, a cohort of 79 patients with SCLE and 58 with SLE was studied and compared concerning clinical presentation, histologic findings and immunoserologic data. SCLE differed from SLE by cutaneous lesions, significantly less frequent kidney involvement (2.5 vs. 48%), serositis (4 vs. 17%) and arthritis (47 vs. 88%), and the presence of anti-dsDNA antibodies (1.2 vs. 34%) and U1-RNP antibodies (1.2 vs. 17%) [19].

The criteria established by the American College of Rheumatology (ACR) [20] include 11 clinical and laboratory features that were originally intended only for the classification of SLE with the purpose of providing some degree of uniformity to the patient populations of clinical studies. However, this classification system characterizes an heterogeneous group of patients with cutaneous manifestations of the disease. A maximum of 20% of all patients with DLE and

30–50% of all patients with SCLE have 4 or more positive ACR criteria, whereas only 2–3 criteria are fulfilled in most cases of CLE [21–23]. If SCLE patients fulfilling 4 or more ACR criteria were compared with SLE patients without these LE-specific skin lesions, remarkable differences would be noted. Photosensitivity was more frequent in patients with SCLE (82%) than in patients without these cutaneous manifestations (45%), whereas arthritis, Raynaud's phenomenon, pleurisy, central nervous system disorder, renal disease, anemia, hypocomplementemia and anti-dsDNA antibodies were significantly more frequent in patients with SLE [24]. However, the ACR criteria assign too much weight to the skin as an expression of a multiorgan disease, and typical skin lesions for differentiating DLE from SCLE are not included in this list. Consequently, patients having a positive antinuclear antibody titer and nothing clinically other than mucocutaneous manifestations can be classified as having SLE. Using this classification system, correct diagnosis and proper evaluation of the course of CLE is difficult, if not impossible. Therefore, it is necessary to select more uniform populations for studies in CLE and SLE and to develop suitable diagnostic criteria for recognizing CLE patients at risk to develop SLE [25, 26].

For elucidating this subject, several studies were performed and classification criteria for CLE have been suggested by the European Academy of Dermatology and Venereology (EADV) to obtain more reliable parameters for classifying these patients [21]. EADV criteria include the ACR criteria and other clinical, histologic and laboratory features that are more suitable for the evaluation of patients with CLE, such as vasculitis of the fingers, muscle weakness, lupus band test, elevated erythrocyte sedimentation rate and anti-Ro/SSA and anti-La/SSB antibodies. In one study, 207 patients with CCLE and SCLE were comparatively classified according to the ACR and EADV criteria. ACR criteria showed a sensitivity of 88%, a specificity of 79%, a positive predictive value of 56% and a negative predictive value of 96%. EADV criteria showed a sensitivity of only 64%, but a specificity of 93%, a positive predictive value of 61% and a negative predictive value of 94% [27]. These data support the view that the ACR criteria are less useful for evaluating patients with CLE and that the selection of EADV criteria has to be improved to obtain a greater rate of sensitivity. Therefore, the ACR classification criteria are currently being reevaluated in the context of a trial that is also including dermatologic control groups such as rosacea, psoriasis, eczema and amyopathic dermatomyositis.

SCLE Lesions Present with an Annular or Papulosquamous Morphology

SCLE is a distinct entity with specific clinical and serologic features that was first described by James N. Gilliam in 1977 [2], with expanded discussion

by Richard Sontheimer in 1979 [28] and 1982 [29]. James N. Gilliam initially hypothesized that patients who present with widespread, nonscarring, photosensitive/photoinducible skin lesions might share common clinical, histologic, laboratory and immunogenetic features and thereby represent a distinctive subset of CLE. However, James N. Gilliam was not the first to observe and describe SCLE skin lesions. Patients exhibiting such lesions had previously been discussed under various designations in the historical literature (lupus marginatus, symmetrical erythema centrifigum, disseminated DLE, autoimmune annular erythema, LE gyratus repens, psoriasiform LE, pityriasiform LE and maculopapular photosensitive LE) [30].

In the following years, it became obvious that SCLE skin lesions can be triggered by UV light and a number of different drugs, such as thiazide diuretics, calcium channel blockers, ACE inhibitors and allylamine antifungals, the majority of which are also capable of independently producing photosensitivity drug reactions. In addition, most patients with this subtype have prominent cutaneous and musculoskeletal complaints, but no more than 10% experience life-threatening complications of SLE in their lifetime [31]. SCLE is further associated with a distinctive immunogenetic background including the production of anti-Ro/SSA antibodies and the 8.1 ancestral haplotype, the common Caucasoid haplotype (HLA-A1, Cw7, B8, TNFAB*a2b3, TNFN*S, C2*C, Bf*s, C4A*Q0, C4B*1, DRB1*0301, DRB3*0101, DQA1*0501, DQB1*0201) that is carried by most people who type for HLA-B8, DR3. This is the same genetic background upon which primary Sjögren's syndrome develops and overlap between SCLE and Sjögren's syndrome has been observed in several studies.

Clinically, SCLE patients present with sharply, demarcated, elevated, erythematous papules or small plaques covered with fine scales affecting shoulders, upper back, extensor aspects of the arms and the V-area of the back or neck. The lesions expand and merge in some patients, producing papulosquamous lesions in retiform arrays that can mimic those of psoriasis vulgaris. In other patients, the primary lesions expand and clear centrally to produce annular lesions that may merge into polycyclic arrays [7, 32]. Both types of SCLE lesions heal without scarring but can leave long-lasting and permanent vitiligo-like pigmentary changes as a clue for the clinical diagnosis [33]. Some groups have observed a predominance of papulosquamous lesions, whereas others have noted an abundance of the annular/polycyclic type [19, 34–39]. It is not obvious why patients preferentially develop either one subtype or the other, as no characteristic feature, such as photosensitivity, anti-Ro/SSA or anti-La/SSB antibodies or musculoskeletal complaints have been associated in a higher incidence with one subtype.

Both subtypes of SCLE skin lesions are thought to result from 4 sequential stages: (1) inheritance of susceptibility genes; (2) loss of tolerance/induction of

autoimmunity; (3) expansion/maturation of autoimmune responses; (4) tissue injury/disease induction resulting from various autoimmune effector mechanisms [30]. TNF- α promoter (−308A) and C1q (*C1QA*-Gly70_{GGG/A}) gene polymorphisms have been suggested to represent SCLE susceptibility genes. Dysregulated clearance of UVB-induced apoptotic keratinocytes has been implicated in the loss of tolerance to autoantigens such as Ro/SSA. In addition, the interaction of anti-Ro/SSA antibodies with cell surface displayed the Ro/SSA antigen on keratinocytes undergoing UVB-induced apoptosis has been implicated as a potential pathogenetic factor in SCLE [40]. In support of this hypothesis is the work by Clancy et al. [41], who recently reported that anti-Ro/SSA antibodies from the maternal circulation can inhibit the physiologic clearance of apoptotic cardiocytes in the development of fetal heart, thereby predisposing to neonatal LE and congenital heart block. However, it currently remains under investigation if one of these stages is more prominent in one of the SCLE skin lesions resulting in an annular or papulosquamous subtype.

Dermal Scarring Exists in DLE but Not in SCLE, ACLE and ICLE

Classical DLE is the most common subtype of CCLE and can occur as a localized process, commonly with lesions above the neck in sun-exposed areas, or as a generalized process with lesions above and below the neck. This disseminated form of DLE, especially when involving the trunk, is particularly associated with an increased risk of progression to SLE [8, 42, 43].

The cutaneous manifestations of patients with DLE begin with flat or slightly elevated, demarcated, erythematous macules or papules with a scaly surface. Early skin lesions most commonly evolve into larger, coin-shaped ('discoid'), confluent, disfiguring plaques of varying size, demonstrating a prominent adherent scale formation [44]. When the adherent scale is peeled back from more advanced lesions, follicle-sized keratotic spikes similar in appearance to carpet tacks can be seen to project from the undersurface of the scale ('the carpet tack sign'). A biopsy is often needed to make the diagnosis, as clinically there are several other diseases that are considered in the differential diagnosis.

Teleangiectasia and hyperpigmentation can replace the active inflammation of DLE lesions, resulting in a poikilodermatous appearance [45]. DLE plaques are generally progressive, and resolution of the skin lesions leaves more or less evident atrophy and scarring, depending on the duration and severity of the lesions during the active phase. This may result in considerable mutilations, particularly when present in acral regions on the face, such as the tip of the nose and the ears, or in irreversible scarring alopecia on the scalp. A characteristic pitted, acneiform scarring is also a common feature of the perioral area [3]. DLE on the scalp may be the only cutaneous manifestation in 10% of cases and thus presents a classic differential diagnosis of scarring alopecia [46]. In some

patients, DLE on the scalp progresses to the point of total, irreversible scarring alopecia and may be accompanied by secondary bacterial superinfection. In contrast, other subtypes of CLE, such as ACLE, SCLE and ICLE, do not show any signs of atrophy or scarring [47, 48]. This might be due to the different course of the disease, as the adjectives acute, subacute and intermittent in these designations conform to the classic dermatologic definitions of these terms.

The localized form of ACLE commonly presents as the classic ‘malar rash’ or ‘butterfly rash’ on the central portion of the face and may only affect the skin transiently preceding the onset of a multisystem disease [9, 49, 50]. The skin lesions begin with small, discrete erythematous macules and papules, occasionally associated with fine scales and gradually becoming confluent and hyperkeratotic. Facial swelling may be severe in some patients with this subtype. The generalized form of ACLE is a less common variety and the onset of this form usually coincides with exacerbation of systemic manifestations developing a prolonged disease activity and may resemble a drug eruption or can simulate toxic epidermal necrolysis. The skin lesions may be located anywhere on the body although the preferred sites are above the waistline [48, 51] and characterized by a symmetrically distributed maculopapular or exanthematous eruption with a pruritic component.

ICLE has recently been defined as a further distinct subtype of CLE, including LET as a separate entity. In several aspects, LET differs from other variants of CLE and scarring, the hallmark of DLE, does not occur in LET, not even in patients with recurrent skin lesions [4]. Follicular plugging and adherent hyperkeratotic scaling, which are further features of DLE, are also not seen in any of the patients with LET. Hypopigmentation, frequently evident in patients with SCLE after the active phase with erythema and scaling, has never been detected in this subtype [52]. The skin lesions of LET are characterized by succulent, urticaria-like, single or multiple papules and plaques with a bright reddish or violaceous smooth surface. The swollen appearance of the lesions and the absence of clinically visible epidermal involvement are the most important features of this subtype. The borders are sharply limited and, in some cases, there is a tendency for the lesions to coalesce in the periphery, producing a gyrate configuration, or to swell in the periphery and flatten in the center, producing an annular configuration [53]. LET lesions can coexist with DLE lesions [54] and have been reported to mimic alopecia areata when present on the scalp [55]. Some patients develop erythematous, annular lesions on the cheeks and upper extremities imitating the annular type of SCLE, and recently a patient with LET following the lines of Blaschko has been described [56]. Anti-Ro/SSA and anti-La/SSB antibodies have been found in less than 10% of the patients with LET. However, association with systemic disease seems to be very rare in patients with LET and has only been documented in very few cases [57].

The molecular and cellular basis for the atrophic dermal scarring that is characteristically produced by DLE disease activity has not been studied. The extended periods of inflammation that are typical of this subtype might contribute to this phenomenon. However, it has been shown that dermal atrophy can appear in DLE lesions as early as 6 weeks after their initial appearance. Furthermore, the atrophy associated with DLE lesions might relate to the deeper pattern of chronic inflammation seen in this disease. Periappendageal inflammation and inflammation in the superficial portion of the underlying subcutaneous tissue is typical of DLE lesions. Chronic subcutaneous inflammation in other settings such as LE panniculitis characteristically leads to saucerized clinical patterns of subcutaneous atrophy. It would be interesting to know how much of the presumed dermal scarring of DLE lesions might in fact be due to a combination of dermal and subcutaneous atrophy.

With rare exceptions, the molecular pathways that are involved in wound healing, such as TGF- β signaling, have not been systematically examined with respect to DLE-associated atrophic scarring. However, Nyberg et al. [58] have reported that cultured fibroblasts from scarring CLE lesions displayed significantly decreased proliferation rates compared to nonscarring CLE lesions and controls. There were no significant differences in levels of IL-6 or TGF- β in supernatants of UV-irradiated fibroblasts from patients compared to controls and IL-4 and the soluble forms of ICAM-1 and VCAM-1 were below detection level. The response to UV irradiation was similar in CLE to that of normal cells in the parameters studied.

Photosensitivity

A Sunburn Does Not Go Away

Abnormal reactivity to UV light is an important factor in the pathogenesis of CLE, and photosensitivity shows a strong association with all disease subtypes [59]. The original concept of photosensitivity in CLE was based on observations made since the beginning of the 19th century. Several articles in the literature documented that in all forms of CLE, skin lesions are found predominantly on sun-exposed areas, such as the face, V-area of the neck and extensor aspects of the arms, and that exposure to sunlight can induce new skin eruptions, exacerbate existing lesions, cause progression of the disease to non-UV-exposed areas or induce systemic activity [60].

However, the term photosensitivity is problematic and poorly defined by the ACR as ‘a skin rash as a result of unusual reaction to sunlight by patient history or physician observation’. This is an extremely broad definition that can be fulfilled by a variety of other diseases, such as polymorphous light eruption

(PLE), photoallergic contact dermatitis and dermatomyositis. In addition, Albrecht et al. [61] recently criticized that malar rash, a further ACR criterion for the classification of SLE, is often indistinguishable from photosensitivity and therefore, both criteria are not independent. In the opinion of these authors, a control group is needed for developing new criteria, which should not only include patients with connective tissue diseases, but also patients with photo-dermatoses such as PLE.

A detailed clinical history is important for the diagnosis and assessment of photosensitivity in patients with CLE; however, a negative history of photosensitivity does not necessarily exclude a sensitivity to sunlight [62]. This might be due to the fact that, in contrast to PLE, the development of UV-induced skin lesions in patients with CLE is characterized by a latency period of up to several weeks. For this reason, a relationship between sun exposure and exacerbation of CLE does not seem obvious to the patient and therefore, it might be difficult for some patients to link sun exposure to their disease. In addition, the occurrence of photosensitivity varies among the different subtypes of CLE and often differs between various ethnic groups, such as African blacks [63]. Walchner et al. [64] have also observed that mainly patients younger than 40 years reported photosensitivity, suggesting that the age at onset of disease also plays a role.

Due to the numerous observations and the clinical evidence demonstrating the clear relationship between sunlight exposure and skin manifestations of CLE, experimental light testing with different wavelengths has been developed to better define UV sensitivity in patients with a photosensitive form of the disease [59]. Results of earlier experimental photoprovocation studies indicated that characteristic skin lesions of CLE could be induced by repeatedly delivering high doses of UVB to the same test site [65, 66]. In 1986, Lehmann et al. [67] developed a standardized protocol and demonstrated that the action spectrum of CLE also reaches into the long-wave UVA region. In the following years, a total of 128 patients were investigated and characteristic skin lesions clinically and histologically compatible with CLE were experimentally induced by UVA or UVB irradiation in 43% of patients [68]. A practical consequence of UVA sensitivity is that patients with CLE are not adequately protected by window glass or by conventional sunscreens, which mostly absorb UVA poorly. Moreover, high-intensity UVA sources in tanning salons might be dangerous for these patients [69, 70].

In the ensuing 15 years, the standardized protocol for phototesting in patients with CLE has been optimized by taking into account several factors, such as light source, test area of irradiated skin, dose of UV exposure and frequency of irradiation [62]. Furthermore, combined UVA and UVB irradiation has been performed and most of the patients developed characteristic skin

lesions using this regimen. Altogether, skin lesions characteristic for CLE were observed in 54% of patients; 42% of these patients reacted to UVB irradiation only and 34% to UVA irradiation only. Interestingly, there were substantial differences in the clinical subtypes of CLE with regard to response to the different UV wavelengths. Patients with LET were found to be the most photosensitive subtype of CLE, since phototesting revealed characteristic skin lesions in 72% of these patients. In contrast, pathologic skin reactions were induced by UV irradiation in 63% of patients with SCLE, in 60% of patients with ACLE and in 45% of patients with DLE. However, it is still unclear why skin lesions cannot be reproduced under the same conditions several months after the initial phototest and why UV irradiation does not show positive results in all patients tested, providing indirect evidence for variant factors in the pathogenesis of CLE. In addition, it is not obvious why UV-induced skin lesions in patients with this disease are characterized by a latency period of 8.0 ± 4.6 days (ranging from 1 day to 3 weeks). Therefore, this testing regimen is not only an optimal procedure to evaluate photosensitivity in CLE patients, the capacity of UVA and UVB irradiation to reproduce skin lesions in this disease is further an ideal model for several experimental approaches, which allow the study of inflammatory and immunologic events that take place prior to and during lesion formation.

Apoptotic Keratinocytes Accumulate after UV Irradiation

Keratinocytes die by apoptosis as part of their normal program of differentiation [71–73]; however, the molecular mechanism controlling this programmed cell death in the epidermis is complex and still poorly understood. Basilar keratinocytes have been found to be relatively resistant to apoptosis induced by a variety of stimuli [74]. It has long been known that suprabasilar keratinocytes are more sensitive to UV-induced apoptotic cell death – such cells were called ‘sunburn cells’ by morphologists [75].

Interestingly, a significant higher number of apoptotic keratinocytes has been found in primary and UV-induced skin lesions of patients with CLE compared with healthy controls [76–78]. Moreover, in the majority of patients with CLE the number of apoptotic nuclei increased significantly between day 1 and day 3 after a single UV exposure, suggesting that late apoptotic cells accumulate in the skin of a large subgroup of patients with this disease [78, 79]. Although detection of a high number of apoptotic cells in the epidermis of CLE patients may reflect an increase in apoptosis, a deficient clearance of apoptotic debris could also lead to the increased number of apoptotic keratinocytes after UV irradiation.

A study in humans has demonstrated that the clearance of apoptotic lymphocytes by macrophages is indeed impaired in some patients with SLE [80],

and impaired uptake of apoptotic cells by macrophages has been noted in germinal centers of these patients [81]. A characteristic feature of the germinal centers is the presence of specialized phagocytes, usually referred to as tingible body macrophages (TBM). Under healthy conditions TBM remove apoptotic cells very efficiently in the early phase of apoptosis. However, in a subgroup of patients with SLE, apoptotic cells accumulate in the germinal centers of the lymph nodes. This may be due to impaired phagocytic activity or caused by the absence of TBM [82]. While a number of cellular signals and receptors for the phagocytosis of apoptotic debris have been identified, the magnitude of a clearance defect in patients with SLE remains unclear. Furthermore, it is unknown if impaired clearance is secondary to a defect in the recognition and the binding of apoptotic particles or in macrophage phagocytosis.

Interestingly, systemic autoimmunity has also been noted in mice deficient for molecules potentially involved in the clearance of apoptotic cells including serum amyloid P, C-Mer, C4, IgM or C1q [for review, see 83]. Serum amyloid P is a member of a family of proteins termed pentraxins that bind to apoptotic cells and then interact directly with phagocyte receptors or with C1q. The surface blebs of apoptotic keratinocytes bind C1q, an early component of the complement cascade [84], suggesting that the clearance of these cells by macrophages that express a C1q cell surface receptor is facilitated [85]. A potential role for C1q in the clearance of apoptotic debris has been suggested in LE by 2 observations: first, mice with C1q deficiency develop an SLE-like disease associated with an accumulation of apoptotic cells in the kidney [86]; second, patients with complete congenital C1q deficiency frequently develop LE-like photosensitive eruptions and SLE at an early age [87]. However, the clearance of UV-induced apoptotic keratinocytes was not observed to be altered in C1q-deficient mice [88].

Furthermore, it is still unclear if the increased number of apoptotic cells in the skin of CLE patients has systemic consequences. There is evidence that the biochemical processes of apoptosis generate novel antigens that are uniquely targeted by autoantibodies. In 1994, Casciola-Rosen et al. [89] showed that the translocation of autoantigens to the cell surface of apoptotic blebs may allow circulating autoantibodies to gain access to these autoantigens, which are usually sequestered inside the cells. Furthermore, this group demonstrated that caspases activated during apoptosis cleave intracellular proteins into fragments that are bound by autoantibodies from patients with SLE [89]. In addition, proteins specifically phosphorylated during stress-induced apoptosis are targeted by antibodies from SLE patient sera [90, 91]. More recently, it has been pointed out that patients with skin disease have autoantibodies that preferentially recognize apoptotic-modified U1-70-kD RNP antigen when compared to patients without cutaneous manifestations [92]. These data provide further evidence that immune recognition of

Table 3. Mediator release by UV irradiation (modified after Lin et al. [121])

Source of mediator	Keratinocyte	Mast cell	Endothelial cell	Langerhans cell
UVA	IL-8 IL-10, IL-12, PGE ₂ , PGF _{2α}		PCI ₂	IL-12
UVB	IL-1α, IL-6, IL-8, IL-10, PGE ₂ , PGF _{2α} , TNF-α, TGF-β, GM-CSF	PGD, LTD4, TNF-α, histamine	PCI ₂ , TNF-α	

PG = Prostaglandin; PC = prostacyclin; LT = leukotriene.

modified forms of self-antigens occurs in CLE and suggest that the processes of apoptotic-derived antigens may participate in the pathogenesis of the disease.

UV Light Causes Induction of Cytokines, Chemokines and Nitric Oxide

UV light can induce the release of inflammatory mediators and cytokines by promoting adhesion molecule display and releasing chemokines to attract inflammatory cells into the skin (table 3) [for review, see 93]. It has been demonstrated that UVB irradiation of primary human keratinocytes, in the presence of proinflammatory cytokines such as IL-1 and TNF-α or IFN-γ, significantly enhances the expression of the inflammatory chemokines CCL5, CCL20, CCL22 and CXCL [94]. This is of relevance in CLE as expression of CCL5 and CXCL8 has been reported to be highly upregulated in skin lesions of patients with this disease. Following phototesting, elevated levels of CCL27, a novel skin-specific chemokine known to recruit memory T cells into the skin, were also found in the dermis of CLE patients. In addition, the CXCR3 ligands CXCL9, CXCL10 and CXCL11 have been identified as the most abundantly expressed genes in patients with this disease [95]. Furthermore, it has been reported that the CCR4 ligand TARC/CCL17 is strongly expressed in skin lesions and elevated in the serum of patients with CLE. The functional relevance of lymphocytic CCR4 expression could be confirmed by TARC/CCL17-specific in vitro migration assays, suggesting that CCR4 and TARC/CCL17 play a role in the pathophysiology of this disease.

Recent gene array studies have demonstrated that type I interferons (IFN-α/β) play an important role in the pathogenesis of SLE. Interestingly, strong

expression of MxA, a protein specifically induced by type I interferons, has been found in lesional skin of patients with CLE and large numbers of infiltrating CXCR3+ lymphocytes correlated closely with lesional MxA expression [96]. Furthermore, natural IFN- α -producing cells, also termed plasmacytoid dendritic cells, have been detected in CLE lesions [97, 98] and are also associated with the presence of MxA [96]. This suggests that local IFN- α production by these cells promotes T helper 1-biased inflammation. Although the pathophysiologic role of skin-infiltrating lymphocytes is undoubted, their recruitment and activation pathways in inflammatory skin diseases are not completely understood.

In addition, UV exposure has been shown to modulate local nitric oxide (NO) production in the skin by the inducible NO synthase (iNOS) [99]. The NO liberated following UV irradiation plays a significant role in initiating melanogenesis, erythema and immunosuppression. New evidence suggests that it may also be involved in protecting keratinocytes against UV-induced apoptosis by increasing Bcl-2 expression and inhibiting UVA-induced overexpression of Bax protein in endothelial cells in vitro [100]. It has further been demonstrated by the same group that the presence of nitrite and not nitrate, during irradiation of endothelial cells, exerts a potent and concentration-dependent protection against UVA-induced apoptotic cell death [100]. Recently, Weller et al. [101] have suggested an anti-apoptotic role for NO in keratinocytes after exposure to UVB. However, when applied to normal, nonirradiated skin, NO induced accumulation of CD4+ and CD8+ T cells, expression of ICAM-1 and VCAM-1, and accumulation of p53, followed by keratinocyte apoptosis [102]. Altered expression of this molecule may therefore provide a further link between dysregulated keratinocyte apoptosis and inflammation. Moreover, it has also been demonstrated that iNOS is expressed in skin lesions of patients with CLE after UVA and UVB irradiation [103]. However, in CLE an iNOS-specific signal appeared not before day 3 after UV exposure in contrast to healthy controls, in which iNOS expression was already present at day 1 after irradiation and no longer expressed at day 3.

These results suggest that the kinetics of iNOS induction and the time span of local iNOS expression might play an important role in the pathogenesis of CLE. According to the evidence of a delayed and prolonged expression of iNOS in the skin of patients with this disease after UV exposure, it needs to be determined whether NO via chemical donors appears to be a promising target for therapeutic intervention in this disease. It has further been reported that NO production is increased in patients with SLE possibly due to upregulated iNOS expression in activated endothelial cells and keratinocytes [104]. In addition, serum nitrite correlated with measures of disease activity and titers of anti-dsDNA antibodies in these patients. However, polymorphism in the iNOS gene

promotor does not seem to play a relevant role in the pathogenesis of patients with SLE [105].

T Cell-Mediated Injury and Influence of Regulatory T Cells

There is growing evidence that the highly specific humoral immune response to autoantigens in SLE is T cell dependent [106]. Although there is no murine model that accurately recapitulates the cutaneous pathology seen in human CLE, murine models have nevertheless been useful in the dissection of the potential cellular mechanisms of autoimmune inflammation. These models include spontaneously occurring and UV-accelerated forms of the disease in MRL/lpr and NZB/NZW mice [for review, see 107]; however, the predominant type of T cells in established inflammatory infiltrates remains controversial.

The study of UV-induced CLE lesions in humans has allowed an analysis of early histologic changes and their evolution [108–111]. In early lesions, this analysis has demonstrated CD4+ T cells predominantly at the dermoepidermal junction associated with rare HLA class II expression by keratinocytes. In primary lesions and late UV-induced lesions, an increased number of CD8+ T cells has been observed, epidermal class II MHC expression was increased, and the number of Langerhans cells was reduced. The decrease in Langerhans cell number might reflect dendritic cell activation and migration into the regional lymph nodes.

In addition, Volc-Platzer et al. [112] have suggested that T cells of a specific $\gamma\delta$ T cell receptor phenotype are preferentially expanded within the infiltrates and proposed that these cells may recognize heat shock proteins, which are induced or translocated in keratinocytes by UV light or stress. More recent work has demonstrated that $\gamma\delta$ T cells play a major role in the pathogenesis of infectious and immune diseases, and increased numbers of $\gamma\delta$ T cells have been found in affected skin from chronic CLE patients [113]. Robak et al. [113] have further observed a significantly increased number of $\gamma\delta$ T cells in normal skin of SLE patients compared to healthy controls and a positive correlation between the percentage of $\gamma\delta$ T lymphocytes in the skin and the activity of SLE.

In the past years, naturally occurring CD4+CD25+ regulatory T cells (Treg) have emerged as an important factor in our understanding of self-tolerance and mechanisms in autoimmune diseases [114]. Recently, a decreased number of peripheral Treg were found in SLE patients compared with healthy controls and a significant correlation could be detected between the number of CD4+CD25+ T cells and disease activity [115]. Lee et al. [116] confirmed these results by showing a significant decrease of Treg in pediatric patients with SLE; however, an inverse correlation between the number of these cells and disease activity as well as autoantibody level was determined in this study. As suggested in a more detailed analysis by Miyara et al. [117], sensitivity of Treg to

CD95L-mediated apoptosis could explain the loss of CD4+CD25+ T cells in patients with active SLE. Interestingly, a significantly reduced level of Treg has also been observed to correlate with autoantibody production and the onset of a lupus-like disease in different murine models [118, 119]. A decrease in Treg has also been observed only at the site of inflammation in skin lesions of CLE. This reduction of Treg in the dermal infiltrate was independent of the disease subtype [120]; however, patients with CLE did not show a general Treg defect as supported by a normal frequency of circulating Treg subpopulation, by their capacity to suppress conventional T cell proliferation, and by a normal sensitivity of Treg towards CD95L-mediated apoptosis. These data suggest an organ-specific abnormality of Treg in CLE skin lesions rather than a global dysfunction as reported for patients with a systemic manifestation of the disease.

Acknowledgment

This work was supported by a research grant from the Deutsche Forschungsgemeinschaft to A.K. (KU 1559/1-2) and by the Richard and Adeline Fleischaker Chair in Dermatology Research at the University of Oklahoma Health Sciences Center to R.D.S.

References

- 1 Sontheimer RD: The lexicon of cutaneous lupus erythematosus – a review and personal perspective on the nomenclature and classification of the cutaneous manifestations of lupus erythematosus. *Lupus* 1997;6:84–95.
- 2 Gilliam JN: The cutaneous signs of lupus erythematosus. *Cont Educ Fam Phys* 1977;6:34–70.
- 3 Costner MI, Sontheimer RD, Provost TT: Lupus erythematosus; in Sontheimer RD, Provost TT (eds): *Cutaneous Manifestations of Rheumatic Diseases*. Philadelphia, Williams & Wilkins, 2003, pp 15–64.
- 4 Kuhn A, Richter-Hintz D, Oslislo C, Ruzicka T, Megahed M, Lehmann P: Lupus erythematosus tumidus – a neglected subset of cutaneous lupus erythematosus: report of 40 cases. *Arch Dermatol* 2000;136:1033–1041.
- 5 Kuhn A, Ruzicka T: Classification of cutaneous lupus erythematosus; in Kuhn A, Lehmann P, Ruzicka T (eds): *Cutaneous Lupus Erythematosus*. Heidelberg, Springer, 2004, pp 53–58.
- 6 Provost TT: Nonspecific cutaneous manifestations of systemic lupus erythematosus; in Kuhn A, Lehmann P, Ruzicka T (eds): *Cutaneous Lupus Erythematosus*. Heidelberg, Springer, 2004, pp 93–106.
- 7 Sontheimer RD: Subacute cutaneous lupus erythematosus: a decade's perspective. *Med Clin North Am* 1989;73:1073–1090.
- 8 Tebbe B, Mansmann U, Wollina U, Auer-Grumbach P, Licht-Mbalyohere A, Arsenmeier M, Orfanos CE: Markers in cutaneous lupus erythematosus indicating systemic involvement: a multicenter study on 296 patients. *Acta Derm Venereol* 1997;77:305–308.
- 9 Watanabe T, Tsuchida T: Classification of lupus erythematosus based upon cutaneous manifestations: dermatological, systemic and laboratory findings in 191 patients. *Dermatology* 1995;190:277–283.
- 10 Ratnoff WD: Inherited deficiencies of complement in rheumatic diseases. *Rheum Dis Clin North Am* 1996;22:75–94.
- 11 Petri M, Watson R, Winkelstein JA, McLean RH: Clinical expression of systemic lupus erythematosus in patients with C4A deficiency. *Medicine (Baltimore)* 1993;72:236–244.

- 12 Sullivan KE: Complement deficiency and autoimmunity. *Curr Opin Pediatr* 1998;10:600–606.
- 13 Kanda N, Tsuchida T, Watanabe T, Tamaki K: Clinical features of systemic lupus erythematosus in men: characteristics of the cutaneous manifestations. *Dermatology* 1996;193:6–10.
- 14 Miller MH, Urowitz MB, Gladman DD, Killinger DW: Systemic lupus erythematosus in males. *Medicine (Baltimore)* 1983;62:327–334.
- 15 Sequeira JF, Keser G, Greenstein B, Wheeler MJ, Duarte PC, Khamashta MA, Hughes GR: Systemic lupus erythematosus: sex hormones in male patients. *Lupus* 1993;2:315–317.
- 16 Font J, Cervera R, Navarro M, Pallares L, Lopez-Soto A, Vivancos J, Ingelmo M: Systemic lupus erythematosus in men: clinical and immunological characteristics. *Ann Rheum Dis* 1992;51:1050–1052.
- 17 Koh WH, Fong KY, Boey ML, Feng PH: Systemic lupus erythematosus in 61 oriental males: a study of clinical and laboratory manifestations. *Br J Rheumatol* 1994;33:339–342.
- 18 Wallace DJ, Pistiner M, Nessim S, Metzger AL, Klinenberg JR: Cutaneous lupus erythematosus without systemic lupus erythematosus: clinical and laboratory features. *Semin Arthritis Rheum* 1992;21:221–226.
- 19 Chlebus E, Wolska H, Blaszczyk M, Jablonska S: Subacute cutaneous lupus erythematosus versus systemic lupus erythematosus: diagnostic criteria and therapeutic implications. *J Am Acad Dermatol* 1998;38:405–412.
- 20 Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–1277.
- 21 Beutner EH, Blaszczyk M, Jablonska S, Chorzelski TP, Vijay K, Wolska H: Studies on criteria of the European Academy of Dermatology and Venereology for the classification of cutaneous lupus erythematosus. *Int J Dermatol* 1991;30:411–417.
- 22 Parodi A, Caproni M, Cardinali C, Bernacchi E, Fuligni A, De Panfilis G, Zane C, Papini M, Veller FC, Vaccaro M, Fabbri P: Clinical, histological and immunopathological features of 58 patients with subacute cutaneous lupus erythematosus: a review by the Italian group of immunodermatology. *Dermatology* 2000;200:6–10.
- 23 Higuchi T, Imamura S, Danno K: Prognosis of discoid lupus erythematosus. *Acta Derm* 1978;73:197–204.
- 24 Lopez-Longo FJ, Monteagudo I, Gonzalez CM, Grau R, Carreno L: Systemic lupus erythematosus: clinical expression and anti-Ro/SS – a response in patients with and without lesions of subacute cutaneous lupus erythematosus. *Lupus* 1997;6:32–39.
- 25 Albrecht J, Taylor L, Berlin JA, Dulay S, Ang G, Fakhrazadeh 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;125:889–894.
- 26 Petri M: Review of classification criteria for systemic lupus erythematosus. *Rheum Dis Clin North Am* 2005;31:245–254.
- 27 Parodi A, Rebora A: ARA and EADV criteria for classification of systemic lupus erythematosus in patients with cutaneous lupus erythematosus. *Dermatology* 1997;194:217–220.
- 28 Sontheimer RD, Thomas JR, Gilliam JN: Subacute cutaneous lupus erythematosus: a cutaneous marker for a distinct lupus erythematosus subset. *Arch Dermatol* 1979;115:1409–1415.
- 29 Gilliam JN, Sontheimer RD: Subacute cutaneous lupus erythematosus. *Clin Rheum Dis* 1982;8:343–352.
- 30 Bano S, Bombardieri S, Doria A, Iaccarino L, Lehmann P, Mosca M, Sontheimer RD, Kuhn A: Lupus erythematosus and the skin. *Clin Exp Rheumatol* 2006;24:S26–S35.
- 31 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;4:253–263.
- 32 Bangert JL, Freemann RG, Sontheimer RD, Gilliam JN: Subacute cutaneous lupus erythematosus and discoid lupus erythematosus: comparative histopathologic findings. *Arch Dermatol* 1984;120:332–337.
- 33 Milde P, Goerz G: Depigmentierung bei Lupus erythematoses (LE) – Klinischer ‘Clue’ zum subakuten kutanen Lupus erythematoses (SCLE). *Z Hautkr* 1994;69:123–126.

- 34 Herrero C, Bielsa I, Font J, Lozano F, Ercilla G, Lecha M, Ingelmo M, Mascaro JM: Subacute cutaneous lupus erythematosus: clinicopathologic findings in thirteen cases. *J Am Acad Dermatol* 1988;19:1057–1062.
- 35 Molad Y, Weinberger A, David M, Garty B, Wysenbeek AJ, Pinkhas J: Clinical manifestations and laboratory data of subacute cutaneous lupus erythematosus. *Isr J Med Sci* 1987;23:278–280.
- 36 Callen JP, Klein J: Subacute cutaneous lupus erythematosus: clinical, serologic, immunogenetic, and therapeutic considerations in seventy-two patients. *Arthritis Rheum* 1988;31:1007–1013.
- 37 Fabbri P, Bernacchi E, Neri R: Subacute cutaneous lupus erythematosus: review of the literature and immunological studies of 11 patients. *G Ital Dermatol Venereol* 1990;125:329–336.
- 38 David-Bajaj KM: What's new in cutaneous lupus syndromes: subacute cutaneous lupus erythematosus. *J Invest Dermatol* 1993;100:2S–8S.
- 39 Cohen MR, Crosby D: Systemic disease in subacute cutaneous lupus erythematosus: a controlled comparison with systemic lupus erythematosus. *J Rheumatol* 1994;21:1665–1669.
- 40 Casciola-Rosen LA, Anhalt G, Rosen A: Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J Exp Med* 1994;179:1317–1330.
- 41 Clancy RM, Neufing PJ, Zheng P, O'Mahony M, Nimmerjahn F, Gordon TP, Buyon JP: Impaired clearance of apoptotic cardiocytes is linked to anti-SSA/Ro and -SSB/La antibodies in the pathogenesis of congenital heart block. *J Clin Invest* 2006;116:2413–2422.
- 42 Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, Domenech I, Aydinli AO, Jedryka-Goral A, de Ramon E, et al: Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. *Medicine (Baltimore)* 1993;72:113–124.
- 43 Hymes SR, Jordon RE: Chronic cutaneous lupus erythematosus. *Med Clin North Am* 1989;73:1055–1071.
- 44 Crowson AN, Magro C: The cutaneous pathology of lupus erythematosus: a review. *J Cutan Pathol* 2001;28:1–23.
- 45 Kuhn A, Sontheimer RD, Ruzicka T: Clinical manifestations of cutaneous lupus erythematosus; in Kuhn A, Lehmann P, Ruzicka T (eds): *Cutaneous Lupus Erythematosus*. Heidelberg, Springer, 2004, pp 59–92.
- 46 Prystowsky SD, Gilliam JM: Discoid lupus erythematosus as part of a larger disease spectrum: correlation of clinical features with laboratory findings in lupus erythematosus. *Arch Dermatol* 1975;111:1448–1452.
- 47 Norden D, Weinberg JM, Schumacher HR, Keenan G, Freundlich B: Bilateral periorbital edema in systemic lupus erythematosus. *J Rheumatol* 1993;20:2158–2160.
- 48 Yell JA, Mbuagbaw J, Burge SM: Cutaneous manifestations of systemic lupus erythematosus. *Br J Dermatol* 1996;135:355–362.
- 49 Wysenbeek AJ, Guedj D, Amit M, Weinberger A: Rash in systemic lupus erythematosus: prevalence and relation to cutaneous and non-cutaneous disease manifestations. *Ann Rheum Dis* 1992;51:717–719.
- 50 Yung A, Oakley A: Bullous systemic lupus erythematosus. *Australas J Dermatol* 2000;41:234–237.
- 51 Sontheimer RD, Provost TT: Lupus erythematosus; in Sontheimer RD, Provost TT (eds): *Cutaneous Manifestations of Rheumatic Diseases*. Baltimore, Williams & Wilkins, 2003, pp 1–71.
- 52 David-Bajaj KM, Davis BM: Pathology, immunopathology, and immunohistochemistry in cutaneous lupus erythematosus. *Lupus* 1997;6:145–157.
- 53 Mascaro JM, Herrero C, Hausmann G: Uncommon cutaneous manifestations of lupus erythematosus. *Lupus* 1997;6:122–131.
- 54 Ruiz H, Sanchez JL: Tumid lupus erythematosus. *Am J Dermatopathol* 1999;21:356–360.
- 55 Werth VP, White WL, Sanchez MR, Franks AG: Incidence of alopecia areata in lupus erythematosus. *Arch Dermatol* 1992;128:368–371.
- 56 Pacheco TR, Spates ST, Lee LA: Unilateral tumid lupus erythematosus. *Lupus* 2002;11:388–391.
- 57 Jolly M, Laumann AE, Shea CR, Utset TO: Lupus erythematosus tumidus in systemic lupus erythematosus: novel association and possible role of early treatment in prevention of discoid lupus erythematosus. *Lupus* 2004;13:64–69.

- 58 Nyberg F, Fransson J, Stephansson E: Proliferation and effects of UVA irradiation in cultured fibroblasts from lesions in cutaneous lupus erythematosus. *Exp Dermatol* 2000;9:53–57.
- 59 Kuhn A, Beissert S: Photosensitivity in lupus erythematosus. *Autoimmunity* 2005;38:519–529.
- 60 Pusey WA: Attacks of lupus erythematosus following exposure to sunlight or other weather factors. *Arch Dermatol Syph* 1915;33:388.
- 61 Albrecht J, Berlin JA, Braverman IM, Callen JP, Connolly MK, Costner MI, Dutz J, Fivenson D, Franks AG, Jorizzo JL, Lee LA, McCauliffe DP, Sontheimer RD, Werth VP: Dermatology position paper on the revision of the 1982 ACR criteria for systemic lupus erythematosus. *Lupus* 2004;13:839–849.
- 62 Kuhn A, Sonntag M, Richter-Hintz D, Oslislo C, Megahed M, Ruzicka T, Lehmann P: Phototesting in lupus erythematosus: a 15-year experience. *J Am Acad Dermatol* 2001;45:86–95.
- 63 Sutej PG, Gear AJ, Morrison RC, Tikly M, de Beer M, Dos Santos L, Sher R: Photosensitivity and anti-Ro (SS-A) antibodies in black patients with systemic lupus erythematosus (SLE). *Br J Dermatol* 1989;28:321–324.
- 64 Walchner M, Messer G, Kind P: Phototesting and photoprotection in LE. *Lupus* 1997;6:167–174.
- 65 Cripps DJ, Rankin J: Action spectra of lupus erythematosus and experimental immunofluorescence. *Arch Dermatol* 1973;107:563–567.
- 66 Freeman RG, Knox JM, Owens DW: Cutaneous lesions of lupus erythematosus induced by monochromatic light. *Arch Dermatol* 1969;100:677–682.
- 67 Lehmann P, Hölzle E, von Kries R, Plewig G: Lichtdiagnostische Verfahren bei Patienten mit Verdacht auf Photodermatosen. *Zentralbl Haut und Geschlechtskr* 1986;152:667–682.
- 68 Lehmann P, Hölzle E, Kind P, Goerz G, Plewig G: Experimental reproduction of skin lesions in lupus erythematosus by UVA and UVB radiation. *J Am Acad Dermatol* 1990;22:181–187.
- 69 Stern RS, Docken W: An exacerbation of SLE after visiting a tanning salon. *JAMA* 1986;255:3120.
- 70 Tronnier H, Petri H, Pierchalla P: UV-provozierte bullöse Hautveränderungen bei systemischem Lupus erythematosus. *Z Hautkr* 1988;154:616–617.
- 71 Weedon D, Strutton G: Apoptosis as the mechanism of the involution of hair follicles in catagen transformation. *Acta Derm Venereol* 1981;61:335–339.
- 72 Boehm I: Apoptosis in physiological and pathological skin. Implications for therapy. *Curr Mol Med* 2006;6:375–394.
- 73 McCall CA, Cohen JJ: Programmed cell death in terminally differentiating keratinocytes: role of endogenous endonuclease. *J Invest Dermatol* 1991;97:111–114.
- 74 Norris DA, Whang K, David-Bajar K, Bennion SD: The influence of ultraviolet light on immunological cytotoxicity in the skin. *Photochem Photobiol* 1997;65:636–646.
- 75 Daniels F Jr, Brophy D, Lobitz WC: Histochemical responses of human skin following ultraviolet irradiation. *J Invest Dermatol* 1961;37:351–357.
- 76 Baima B, Sticherling M: Apoptosis in different cutaneous manifestations of lupus erythematosus. *Br J Dermatol* 2001;144:958–966.
- 77 Chung JH, Kwon OS, Eun HC, Youn JI, Song YW, Kim JG, Cho KH: Apoptosis in the pathogenesis of cutaneous lupus erythematosus. *Am J Dermatopathol* 1998;20:233–241.
- 78 Pablos JL, Santiago B, Galindo M, Carreira PE, Ballestin C, Gomez-Reino JJ: Keratinocyte apoptosis and p53 expression in cutaneous lupus and dermatomyositis. *J Pathol* 1999;188:63–68.
- 79 Kuhn A, Herrmann M, Kleber S, Beckmann-Welle M, Fehsel K, Martin-Villalba A, Lehmann P, Ruzicka T, Krammer PH, Kolb-Bachofen V: Accumulation of apoptotic cells in the epidermis of patients with cutaneous lupus erythematosus after ultraviolet irradiation. *Arthritis Rheum* 2006;54:939–950.
- 80 Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR: Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. *Arthritis Rheum* 1998;41:1241–1250.
- 81 Baumann I, Kolowos W, Voll RE, Manger B, Gaipl U, Neuhuber WL, Kirchner T, Kalden JR, Herrmann M: Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis Rheum* 2002;46:191–201.

- 82 Gaipf US, Kuhn A, Sheriff A, Munoz LE, Franz S, Voll RE, Kalden JR, Herrmann M: Clearance of apoptotic cells in human SLE. *Curr Dir Autoimmun* 2006;9:173–187.
- 83 Roos A, Xu W, Castellano G, Nauta AJ, Garred P, Daha MR, van Kooten C: Mini-review: a pivotal role for innate immunity in the clearance of apoptotic cells. *Eur J Immunol* 2004;34:921–929.
- 84 Korb LC, Ahearn JM: C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: complement deficiency and systemic lupus erythematosus revisited. *J Immunol* 1997;158:4525–4528.
- 85 Nepomuceno RR, Henschen-Edman AH, Burgess WH, Tenner AJ: cDNA cloning and primary structure analysis of C1qR_p, the human C1q/MBL/SPA receptor that mediates enhanced phagocytosis in vitro. *Immunity* 1997;6:119–129.
- 86 Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, Loos M, Pandolfi PP, Walport MJ: Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Genet* 1998;19:56–59.
- 87 Bowness P, Davies KA, Norsworthy PJ, Athanassiou P, Taylor-Wiedeman J, Borysiewicz LK, Meyer PA, Walport MJ: Hereditary C1q deficiency and systemic lupus erythematosus. *Qjm* 1994;87:455–464.
- 88 Pickering MC, Fischer S, Lewis MR, Walport MJ, Botto M, Cook HT: Ultraviolet-radiation-induced keratinocyte apoptosis in C1q-deficient mice. *J Invest Dermatol* 2001;117:52–58.
- 89 Casciola-Rosen LA, Anhalt GJ, Rosen A: DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. *J Exp Med* 1995;182:1625–1634.
- 90 Utz PJ, Hottelet M, Schur PH, Anderson P: Proteins phosphorylated during stress-induced apoptosis are common targets for autoantibody production in patients with systemic lupus erythematosus. *J Exp Med* 1997;185:843–854.
- 91 Utz PJ, Anderson P: Posttranslational protein modifications, apoptosis, and the bypass of tolerance to autoantigens. *Arthritis Rheum* 1998;41:1152–1160.
- 92 Greidinger EL, Casciola-Rosen L, Morris SM, Hoffman RW, Rosen A: Autoantibody recognition of distinctly modified forms of the U1–70-kD antigen is associated with different clinical disease manifestations. *Arthritis Rheum* 2000;43:881–888.
- 93 Bennion SD, Norris DA: Ultraviolet light modulation of autoantigens, epidermal cytokines and adhesion molecules as contributing factors of the pathogenesis of cutaneous LE. *Lupus* 1997;6:181–192.
- 94 Meller S, Winterberg F, Gilliet M, Muller A, Lauceviciute I, Rieker J, Neumann NJ, Kubitz A, Gombert M, Bunemann E, Wiesner U, Franken-Kunkel P, Kanzler H, Dieu-Nosjean MC, Amara A, Ruzicka T, Lehmann P, Zlotnik A, Homey B: Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment: an amplification cycle triggering cutaneous lupus erythematosus. *Arthritis Rheum* 2005;52:1504–1516.
- 95 Wenzel J, Henze S, Worenkamper E: Role of the chemokine receptor CCR4 and its ligand thymus- and activation-regulated chemokine/CCL 17 for lymphocyte recruitment in cutaneous lupus erythematosus. *J Invest Dermatol* 2005;124:1241–1248.
- 96 Wenzel J, Worenkamper E, Freutel S, Henze S, Haller O, Bieber T, Tuting T: Enhanced type I interferon signalling promotes Th1-biased inflammation in cutaneous lupus erythematosus. *J Pathol* 2005;205:435–442.
- 97 Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL: Plasmacytoid dendritic cells (natural interferon- α/β -producing cells) accumulate in cutaneous lupus erythematosus lesions. *Am J Pathol* 2001;159:237–243.
- 98 Wollenberg A, Wagner M, Gunther S, Towarowski A, Tuma E, Moderer M, Rothenfusser S, Wetzel S, Endres S, Hartmann G: Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. *J Invest Dermatol* 2002;119:1096–1102.
- 99 Cals-Grierson MM, Ormerod AD: Nitric oxide function in the skin. *Nitric Oxide* 2004;10:179–193.
- 100 Suschek CV, Krischel V, Bruch-Gerharz D, Berendji D, Krutmann J, Kroncke KD, Kolb-Bachofen V: Nitric oxide fully protects against UVA-induced apoptosis in tight correlation with Bcl-2 up-regulation. *J Biol Chem* 1999;274:6130–6137.

- 101 Weller R, Schwentker A, Billiar TR, Vodovotz Y: Autologous nitric oxide protects mouse and human keratinocytes from ultraviolet B radiation-induced apoptosis. *Am J Physiol Cell Physiol* 2003;284:C1140–C1148.
- 102 Ormerod AD, Copeland P, Hay I, Husain A, Ewen SW: The inflammatory and cytotoxic effects of a nitric oxide releasing cream on normal skin. *J Invest Dermatol* 1999;113:392–397.
- 103 Kuhn A, Fehsel K, Lehmann P, Krutmann J, Ruzicka T, Kolb-Bachofen V: Aberrant timing in epidermal expression of inducible nitric oxide synthase after UV irradiation in cutaneous lupus erythematosus. *J Invest Dermatol* 1998;111:149–153.
- 104 Belmont HM, Levartovsky D, Goel A, Amin A, Giorno R, Rediske J, Skovron ML, Abramson SB: Increased nitric oxide production accompanied by the up-regulation of inducible nitric oxide synthase in vascular endothelium from patients with systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1810–1816.
- 105 Lopez-Nevot MA, Ramal L, Jimenez-Alonso J, Martin J: The inducible nitric oxide synthase promoter polymorphism does not confer susceptibility to systemic lupus erythematosus. *Rheumatology (Oxford)* 2003;42:113–116.
- 106 Burlingame RW, Rubin RL, Balderas RS, Theofilopoulos AN: Genesis and evolution of antichromatin autoantibodies in murine lupus implicates T-dependent immunization with self antigen. *J Clin Invest* 1993;91:1687–1696.
- 107 Furukawa F: Animal models of cutaneous lupus erythematosus and lupus erythematosus photosensitivity. *Lupus* 1997;6:193–202.
- 108 Kind P, Lehmann P, Plewig G: Phototesting in lupus erythematosus. *J Invest Dermatol* 1993;100:53S–57S.
- 109 Sontheimer RD, Bergstresser PR: Epidermal Langerhans cell involvement in cutaneous lupus erythematosus. *J Invest Dermatol* 1982;79:237–243.
- 110 Andrews BS, Schenk A, Barr R, Friou G, Mirick G, Ross P: Immunopathology of cutaneous human lupus erythematosus defined by murine monoclonal antibodies. *J Am Acad Dermatol* 1986;15:474–481.
- 111 Shiohara T, Moriya N, Tanaka Y, Arai Y, Hayakawa J, Chiba M, Nagashima M: Immunopathologic study of lichenoid skin diseases: correlation between HLA-DR-positive keratinocytes or Langerhans cells and epidermotropic T cells. *J Am Acad Dermatol* 1988;18:67–74.
- 112 Volc-Platzter B, Anegg B, Milota S, Pickl W, Fischer G: Accumulation of $\gamma\delta$ T cells in chronic cutaneous lupus erythematosus. *J Invest Dermatol* 1993;100:84S–91S.
- 113 Robak E, Niewiadomska H, Robak T, Bartkowiak J, Blonski JZ, Wozniacka A, Pomorski L, Sysa-Jedrezejowska A: Lymphocytes $\gamma\delta$ in clinically normal skin and peripheral blood of patients with systemic lupus erythematosus and their correlation with disease activity. *Mediators Inflamm* 2001;10:179–189.
- 114 Sakaguchi S: Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005;6:345–352.
- 115 Crispin JC, Martinez A, Alcocer-Varela J: Quantification of regulatory T cells in patients with systemic lupus erythematosus. *J Autoimmun* 2003;21:273–276.
- 116 Lee JH, Wang LC, Lin YT, Yang YH, Lin DT, Chiang BL: Inverse correlation between CD4+ regulatory T-cell population and autoantibody levels in paediatric patients with systemic lupus erythematosus. *Immunology* 2006;117:280–286.
- 117 Miyara M, Amoura Z, Parizot C, Badoual C, Dorgham K, Trad S, Nochy D, Debre P, Piette JC, Gorochov G: Global natural regulatory T cell depletion in active systemic lupus erythematosus. *J Immunol* 2005;175:8392–8400.
- 118 Monk CR, Spachidou M, Rovis F, Leung E, Botto M, Lechler RI, Garden OA: MRL/Mp CD4+,CD25– T cells show reduced sensitivity to suppression by CD4+,CD25+ regulatory T cells in vitro: a novel defect of T cell regulation in systemic lupus erythematosus. *Arthritis Rheum* 2005;52:1180–1184.
- 119 Wu HY, Staines NA: A deficiency of CD4+CD25+ T cells permits the development of spontaneous lupus-like disease in mice, and can be reversed by induction of mucosal tolerance to histone peptide autoantigen. *Lupus* 2004;13:192–200.

- 120 Franz B, Fritzsching B, Riehl A, Klemke D, Oberle N, Sykora J, Hartmann M, Enk A, Ruzicka T, Krammer PH, Suri-Payer E, Kuhn A: Low number of regulatory T cells in skin lesions of patients with cutaneous lupus erythematoses. *Arthritis Rheum* 2007;56:1910–1920.
- 121 Lin JH, Sontheimer RD, Werth VP: Pathophysiology of cutaneous lupus erythematosus. *Clin Rev Allergy Immunol* 2007; in press.

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Bullous Pemphigoid and Related Subepidermal Autoimmune Blistering Diseases

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Abstract

The pemphigoid group of autoimmune blistering diseases includes distinct entities (bullous pemphigoid, mucous membrane pemphigoid, pemphigoid gestationis, linear IgA dermatosis and lichen planus pemphigoides) that are characterized by relatively consistent clinical, histologic and immunopathologic findings. Patients with these disorders have anti-basement membrane autoantibodies that often display pathogenic (blister-forming) activity following passive transfer to experimental animals. Interestingly, such autoantibodies target important structural proteins that promote adhesion of epidermis to epidermal basement membrane in human skin. Autoimmune blistering diseases are characterized by substantial morbidity (for example pruritus, pain, disfigurement) and in some instances mortality. Treatment with systemic immunosuppressives has reduced morbidity and mortality in patients with these diseases.

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The stratified squamous epithelium of human epidermis forms a continuous barrier against the external environment. The pathophysiology of blistering diseases illustrates how impairments in epithelial adhesion lead to disorders characterized by substantial morbidity and/or mortality. Blistering diseases can be inherited or acquired; most examples of the latter are autoimmune in nature and characterized by autoantibodies that target adhesion proteins promoting either cell-cell or cell-matrix adhesion in skin. Translational research over the past 25 years has demonstrated that patients with bullous pemphigoid (BP) and its variants have autoantibodies that target specific antigens in normal human skin, that such autoantigens often represent components of adhesion units, and that mutations in genes encoding such proteins are

responsible for inherited diseases characterized by skin fragility and blister formation [1, 2]. Autoantibodies from these patients have been used to identify these autoantigens and demonstrate that they represent important structural proteins in epidermal basement membrane (BM). In the case of selected disorders, passive transfer of IgG (either patient or experimental) against such autoantigens to animals has been shown to induce blisters with the same clinical, histologic and immunopathologic features as those seen in patients. Among these disorders, BP represents the prototypic example of an autoimmune subepidermal blistering disease (table 1). Patients with BP have circulating autoantibodies against two proteins in hemidesmosomes (HDs): BP antigen 1 (BPAG1, also known as BP230) and BP antigen 2 (BPAG2, also known as BP180). In this chapter, we review the biology of human epidermal BM, experimental models of pemphigoid, and the clinical as well as immunopathological features of BP and its variants [mucous membrane pemphigoid (MMP), pemphigoid gestationis (PG), linear IgA dermatosis (LAD) and lichen planus pemphigoides (LPP)].

The Biology of the Epidermal BM

The Hemidesmosomal Complex

The epidermal BM is a highly specialized structure that contains numerous tissue-specific elements (fig. 1) [3]. The first characterization of this ultrastructural region in the skin was made by electron microscopists who identified its four distinct subregions: (1) the cytoskeleton, HDs and plasma membranes of basal keratinocytes; (2) an underlying electron lucent region termed the lamina lucida; (3) the lamina densa (or BM proper); (4) a sublamina densa region in the papillary dermis. The epidermal BM is comprised of proteins derived from keratinocytes of ectodermal origin as well as fibroblasts of mesodermal origin. For example, many proteins (plectin, BPAG1, BPAG2, integrin subunit α_6 and integrin subunit β_4), types IV and VII collagen, laminins 5 ($\alpha 3\beta 3\gamma 2$) and 6 ($\alpha 3\beta 1\gamma 1$), and heparan sulfate proteoglycans are produced by keratinocytes and incorporated within the epidermal BM [4]. In contrast, type VII collagen in anchoring fibrils is made by both keratinocytes and dermal fibroblasts.

The anchoring complex, also known as the hemidesmosomal complex, is a highly specialized structural component of stratified epithelia that mediates the attachment of basal epithelial cells to the underlying BM. At the center of the anchoring complex is the hemidesmosomal plaque, a membrane-associated plate-shaped structure that functions as the primary link between the keratin intermediate filament cytoskeletal network and transmembrane adhesion

Table 1. Clinical forms of pemphigoid and their characteristic features

	Bullous pemphigoid	Mucous membrane pemphigoid	Pemphigoid gestations	Linear IgA disease/chronic bullous disease of childhood	Lichen planus pemphigoides
Clinical features	Tense blisters on inflamed or noninflamed skin; pruritus of variable severity	Erosions, blisters and scars	Urticarial plaques and/or tense blisters during pregnancy and/or puerperium; pruritus typically severe	Pruritic papulovesicular eruption in ‘cluster of jewels’ configuration	Tense blisters on lichenoid lesions or unaffected skin
Distribution	Flexural surfaces and trunk; sometimes oral mucosa	Mucosae of the mouth, eyes, nose, larynx, esophagus or anogenital regions	Umbilical and periumbilical regions; trunk and extremities	Trunk, extremities, abdomen and perineum	Extremities and trunk
Approximate annual incidence	~7 cases per million	~1 case per million	~0.5 cases per million	~0.5 cases per million	Rare, incidence undefined
Histology	Eosinophil-rich subepidermal blisters	Subepidermal blisters containing mononuclear cells and granulocytes; lamellar fibrosis in upper dermis	Eosinophil-rich subepidermal blisters	Subepidermal blisters with papillary dermal microabscesses filled with neutrophils	Lichenoid infiltrate in superficial dermis and eosinophil-rich subepidermal blisters

Table 1. (continued)

	Bullous pemphigoid	Mucous membrane pemphigoid	Pemphigoid gestations	Linear IgA disease/chronic bullous disease of childhood	Lichen planus pemphigoides
Immunopathology	Continuous deposits of IgG and/or C3 in epidermal BM	Continuous deposits of IgG, IgA and/or C3 in epithelial BM	Continuous deposits of C3 in epithelial BM	Continuous deposits of IgA in epithelial BM	Continuous deposits of IgG and/or C3 in epidermal BM
Autoantigens	BPAG1 and BPAG2	BPAG2, laminin 5 and others	BPAG1 and BPAG2	LAD-1 and perhaps other autoantigens	BPAG1 and BPAG2
Prognosis	Chronic course marked by exacerbations and remissions	Chronic course with potential irreversible scarring of mucosal surfaces	Resolution post-partum; likely recurrence with pregnancy; no long-term adverse effects	Chronic; occasional spontaneous remissions; averages ~ 2 years.	Chronic course marked by exacerbations and remissions
Therapy	Systemic glucocorticoids and/or immunosuppressives	Systemic glucocorticoids (plus immunosuppressives in patients with involvement of key mucosal surfaces)	Symptomatic treatment and/or systemic glucocorticoids	Dapsone, sulfapyridine and perhaps systemic glucocorticoids (plus immunosuppressives in severe cases)	Systemic glucocorticoids and/or immunosuppressives

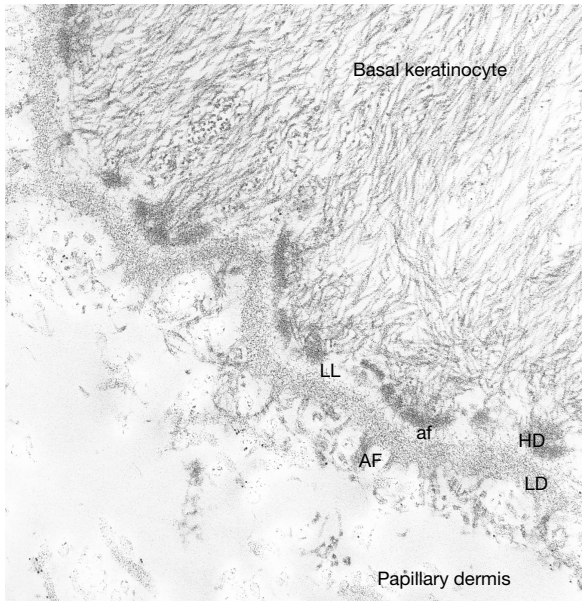


Fig. 1. Electron micrograph of epidermal BM. Electron microscopy studies demonstrate a basal keratinocyte overlying the lamina lucida (LL), which in turn is positioned just above the lamina densa (LD). Ultrastructurally, HD-anchoring filament (af) complexes bind the keratin intermediate filament cytoskeleton of basal keratinocytes to the underlying lamina densa, anchoring fibrils (AF), and fibrillar elements (that is, interstitial collagens and elastin fibers) within the papillary dermis.

molecules located along the base of basal keratinocytes. Ultrastructurally, HDs appear as small electron dense domains (less than $0.5\ \mu\text{m}$) on the ventral surface of plasma membranes in basal keratinocytes. Their most conspicuous component is a tripartite plaque, to which bundles of keratin intermediate filaments are attached superiorly. HDs are associated with a subbasal dense plate in the lamina lucida and are connected via fine thread-like anchoring filaments to the underlying lamina densa. In turn, the latter is anchored to the papillary dermis by cross-banded anchoring fibrils. These various morphological structures (that is intermediate filaments, HDs, anchoring filaments and anchoring fibrils) constitute a functional unit named the hemidesmosomal adhesion complex. This complex promotes stable adhesion of basal keratinocytes to the underlying papillary dermis and also provides signals about the adjacent microenvironment.

BP Antigen 1

Circulating autoantibodies from patients with BP were used to define two autoantigens that reside in the HDs of basal keratinocytes. The first of these autoantigens characterized was BPAG1 [5]. BPAG1 is a 230-kD noncollagenous protein localized to the cytoplasmic plaque of HDs. BPAG1 is a plakin and, like other proteins of this family, interacts with intermediate filament proteins via motifs in its carboxyl terminus. In this manner, BPAG1 promotes adhesion of keratin intermediate filaments to HDs in plasma membranes of basal keratinocytes. BPAG1 has a central coiled-coil α -helical domain; its amino terminus associates with the cytoplasmic domain of BPAG2, integrin subunit β_4 and ERBIN (a protein that interacts with the transmembrane tyrosine kinase receptor Erb-B2).

Consistent with the role that BPAG1 plays in the organization of cytoskeletal intermediate filaments, BPAG1 knockout mice show signs of epithelial fragility in basal keratinocytes. Unexpectedly, these mice also demonstrate neurologic impairment characterized by dystonia and ataxia. Neurologic manifestations in BPAG1 knockout mice develop as a consequence of concomitant inactivation of neuronal isoforms of BPAG1 referred to as nBPAG1 (or dystonin). Neuronal isoforms of BPAG1 contain either actin- or microtubule-binding domains that maintain the cytoarchitecture of neurons.

BP Antigen 2

In 1986, Labib et al. [6] demonstrated by Western blotting that IgG autoantibodies from patients with BP react with a 180-kD epidermal keratinocyte-derived protein. Isolation of the first BP180 cDNA clone, reported by Diaz et al. [7] in 1990, was accomplished by immunoscreening a human keratinocyte cDNA expression library using sera from patients with BP. The human gene encoding BPAG2, designated *COL17A1* (National Center for Biotechnology Information UniGene database No. Hs.117938), has been localized to the long arm of chromosome 10 (map position 10q24.3). Cloning and characterization of the human *COL17A1* gene revealed that it is rather large with a complex organization, comprising 56 exons spanning approximately 52 kb of the genome.

BPAG2 is a type II transmembrane collagen (specifically, type XVII collagen) associated with HD-anchoring filament complexes in basal keratinocytes [8]. The cytoplasmic amino terminus of BPAG2 consists of approximately 500 amino acid residues, containing several potential phosphorylation sites within its central portion. Immunoelectron microscopy studies indicate that BPAG2 spans the lamina lucida and inserts into the lamina densa. The extracellular domain of BPAG2 contains 15 interrupted domains of Gly-X-Y repeating amino acid sequences, characteristic of proteins in the colla-

gen family. Following the classic collagen nomenclature, the collagenous (COL) and noncollagenous (NC) domains of BPAG2 are numbered starting at the carboxyl terminus. Thus, the last and longest collagen domain is designated COL15 and the longest noncollagen stretch, which extends from COL15 to the amino terminus, is designated NC16. NC16 has been further subdivided into extracellular (NC16A), transmembrane (NC16B) and intracellular (NC16C) portions. Rotary shadowing of purified BPAG2 images its cytoplasmic region as a globular head and its extracellular region as a central rod with a flexible tail. These regions are thought to correspond to COL15 and COL1–14, respectively. Recent immunoelectron-microscopic studies suggest that the COL15 rod domain of BPAG2 inserts into the lamina densa and that its COL1–14 carboxyl terminus then loops back through the lamina densa into the lamina lucida.

Many transmembrane proteins, including cell adhesion molecules, growth factors and cytokine receptors, are subjected to limited proteolysis, giving rise to soluble forms that comprise large portions of the extracellular domain of the respective precursor. Similarly, BPAG2 occurs in two forms. One form corresponds to the 180-kD full-length protein; the other corresponds to a soluble, proteolytically processed 120-kD protein corresponding to most of BPAG2's extracellular domain. The processed form of BPAG2 is shed from plasma membranes of basal keratinocytes following proteolysis by members of the furin and ADAM (a disintegrin and metalloproteinase) protein families. This processed protein retains the triple helical structure that is seen in the full-length form of BPAG2. Interestingly, it also contains neoepitopes that are recognized by autoantibodies from patients with LAD. This finding explains the observation that sera from patients with LAD react with the 120-kD processed form of BPAG2, yet fail to react with full-length BPAG2.

BPAG2 is targeted by autoantibodies from patients with BP, MMP, PG, LAD and LLP [9]. Autoantibodies from patients with BP, PG and LAD typically target the NC16A domain of BPAG2, whereas those from patients with MMP tend to target the former as well as the distal carboxyl terminus of BPAG2 (the latter reactivity is thought to account for the likelihood of lesions in such patients to scar). Although not discussed in this chapter, patients with one form of junctional epidermolysis bullosa (EB) – specifically the non-Herlitz subtype (previously named generalized atrophic benign EB; OMIM 113811) – typically possess null mutations in the gene encoding BPAG2. Patients with this disorder characteristically demonstrate complete lack of BPAG2 in their epidermal BMs along with a tendency for skin fragility, subepidermal blister formation, alopecia, dystrophic nails, and dental enamel hypoplasia.

Bullous Pemphigoid

Epidemiology

BP is a chronic blistering disease predominantly seen in the elderly, though there are reports of BP occurring in children as well. There is no known ethnic, racial or gender predilection for BP. The incidence of BP is estimated to be 7 cases per million people per year. The major histocompatibility complex (MHC) class II allele HLA-DQB1*0301 is prevalent in patients with BP.

Clinical Features

Though BP is a polymorphic skin disease, lesions usually consist of tense blisters (1–3 cm) situated on either inflamed or noninflamed skin (fig. 2). Lesions tend to predominate on the lower trunk, axilla, groin and flexor surfaces of the extremities; oral mucosal lesions are present in 10–40% of patients. Sometimes erythema predominates. Patients may present with urticarial lesions, especially early in the course of disease. Such lesions may be rimmed with vesicles and bullae that expand peripherally and progress to confluence. Resolution usually occurs in the center of such lesions and may be accompanied by postinflammatory hyperpigmentation. As lesions evolve, tense blisters tend to rupture and be replaced by flaccid lesions or erosions with or without surmounting crust. Nontraumatized (for example nonexcoriated) lesions tend to heal without scarring. Pruritus is a common feature of BP; it may be mild or quite severe. BP is typically a chronic disease characterized by periods of exacerbation and partial remission. Some patients experience complete remission after 6–10 years of active disease. Despite isolated reports, several studies have shown that patients with BP do not have an increased incidence of malignancy in comparison with appropriately age- and gender-matched controls. The designation ‘localized BP’ refers to patients with disease restricted to relatively few sites. Localized BP most commonly affects the legs; disease in these patients may progress to generalized BP or may remain localized for years. Localized childhood pemphigoid occurs on the vulvar and perivulvar area of girls. As in other forms of localized BP, antibodies from these patients bind characteristic BP autoantigens. In a nonbullous form of pemphigoid, patients have only urticarial and eczematous lesions without blisters. Rare patients with BP may have prurigo nodularis-like or vegetating lesions. Finally, in the rare dyshidrosiform BP, persistent vesiculobullous eruptions occur on the palms and soles and may resemble pompholyx. Such vesiculobullous lesions may be hemorrhagic. During the course of this form of BP, erythematous papular and bullous lesions may develop elsewhere.

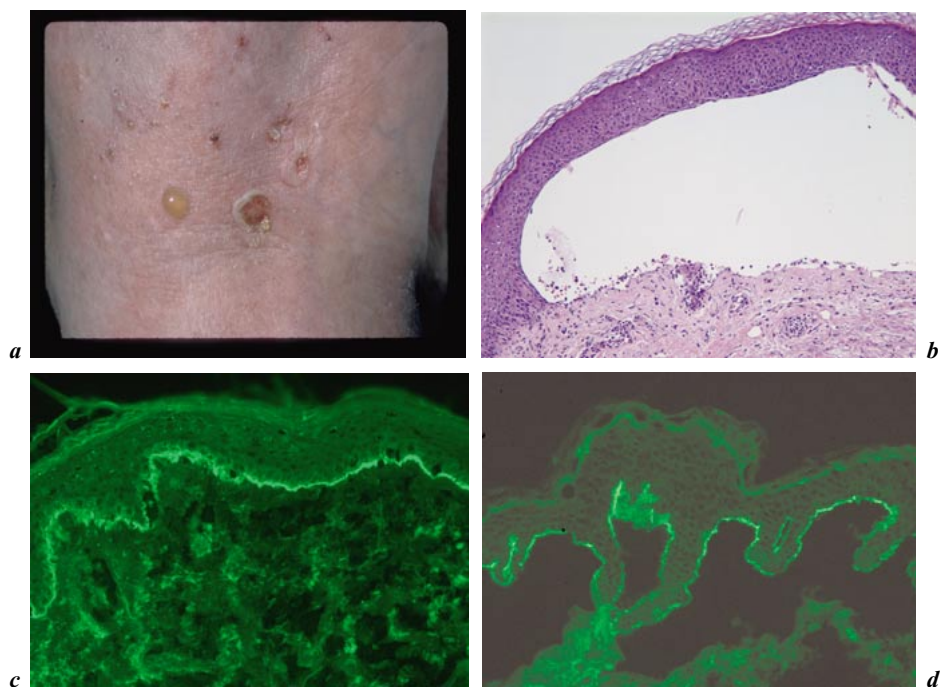


Fig. 2. Clinical, histologic and immunopathologic features of BP. **a** Tense blisters situated on inflamed skin along with crusted erosions in a patient with BP. **b** Light microscopy of lesional skin from a patient with BP demonstrates a subepidermal blister with fibrin and a relatively sparse leukocytic infiltrate. Hematoxylin and eosin stain. **c** Direct immunofluorescence microscopy of perilesional skin from a patient with BP demonstrates continuous linear deposits of IgG in epidermal BM. **d** Indirect immunofluorescence microscopy demonstrates the reactivity of circulating IgG from a patient with BP against the epidermal side of 1 M NaCl split skin.

Pathology

Biopsies of newly developed lesions from patients with BP typically show mast cell degranulation, dermal edema, and an eosinophil-rich leukocytic infiltrate within the papillary dermis and along the epidermal BM (fig. 2). Such alterations typically progress to frank subepidermal blisters. The relative intensity of the leukocytic infiltrate in such lesions generally correlates with their clinical character (that is, lesions on noninflamed skin are relatively ‘cell poor’). Neutrophils may comprise a portion of the leukocytic infiltrate in lesional skin from patients with BP.

Immunopathology

Direct immunofluorescence (IF) microscopy of normal-appearing perilesional skin from patients with BP shows linear deposits of IgG and/or C3 in epidermal BM (fig. 2). The sera of approximately 70% of these patients contain circulating IgG autoantibodies that bind epidermal BM in normal human skin in indirect IF microscopy studies. An even higher percentage of patients show reactivity to the epidermal side of 1 M NaCl split skin, an alternative IF microscopy test substrate that is commonly used to distinguish circulating IgG anti-BM autoantibodies in patients with BP from those in patients with similar, yet different, subepidermal blistering diseases [such as EB acquisita (EBA)]. Infrequently, IgM, IgE or IgA can be found in association with IgG in the epidermal BM of skin from patients with BP. Most patients with circulating anti-BM IgG also have anti-BM IgE in their sera. Correlations between autoantibody levels and disease activity are more commonly observed in serologic testing using an NC16A-specific ELISA rather than routine indirect IF microscopy.

Pathophysiology

Approximately 40 years ago, seminal studies by Beutner, Jordon and colleagues demonstrated that patients with BP have in situ deposits of IgG and complement components in their epidermal BMs as well as circulating IgG autoantibodies that bind the epidermal BM in normal human skin [2]. These findings demonstrated that patients with BP, like those with other autoimmune diseases, have an immune response to constituents of normal tissue (not novel determinants present only in diseased skin). Moreover, because such autoantibodies are not present in patients with other blistering diseases, they were recognized as markers for patients with BP. The studies by Stanley et al. [5], Diaz et al. [7] and others showed that circulating IgG autoantibodies in patients with BP bind 230- and 180-kD proteins associated with HDs in basal keratinocytes (BPAG1 and BPAG2, respectively). That virtually all patients with BP have autoantibodies against an intracellular protein in basal keratinocytes was initially considered to be a paradox. Currently, autoantibodies to BPAG1 are thought to develop as a consequence of keratinocyte injury and determinant spreading of the autoimmune response. These autoantibodies are thought to predominate because the corresponding intracellular autoantigen is not exposed (that is, accessible) to circulating IgG under ordinary circumstances. Although experimental studies have shown that anti-BPAG1 IgG can enhance inflammatory reactions at sites of injured keratinocytes in vivo [10], the autoantigen currently thought to harbor the key pathogenic epitope responsible for initiation and propagation of this disease is BPAG2. Epitope mapping studies of bacterial recombinant proteins show that IgG from most patients with

BP binds a determinant within the first plasma membrane-proximal noncollagenous segment of the BPAG2 ectodomain (that is, its so-called NC16A domain) [11].

Recent studies have shown that T helper (Th) cells and IgG autoantibodies from patients with BP recognize similar or identical epitopes in distinct regions of the BPAG2 ectodomain. Specifically, the majority of autoreactive Th2, Th1 and B cells recognized epitopes within the NH₂-terminal portion, followed by reactivity against the COOH-terminal and central portions of the BPAG2 ectodomain. Of note, T and B cell reactivity against the NH₂-terminal portion of the BPAG2 ectodomain was associated with severe BP (that is, widespread blisters and erosions), while the central portion was more frequently recognized in patients with limited disease (that is, few blisters and erosions). In contrast, less than 50% of the BP patients studied showed a combined T and B cell response against the COOH- and NH₂-terminal globular domains of BPAG1 [12].

Autoreactive T cells in patients with BP produce both Th1 and Th2 cytokines. Th cells are presumably involved in early disease development and perpetuation of acquired autoantibody-mediated bullous diseases, although the exact molecular mechanisms of specific T cell activation in BP remain elusive. Upon proper costimulation, Th cells become activated and secrete distinct cytokines, which stimulate B cells and thus foster plasma cell development and autoantibody production. The initial production of vesicles appears to favor Th1 involvement with the production of complement-fixing IgG₁, while chronicity seems to favor Th2 involvement with the production of IgG₄, IgE and cytokines such as interleukin (IL)-4, IL-5 and IL-13. Antigen-specific degranulation of basophils and/or mast cells from BP patients suggests a mechanism by which IgE may contribute to lesion development.

Circulating IgE levels are often elevated in patients with BP. Several reports suggested that the IgE autoantibodies primarily target BPAG1 or the intracellular domain of BPAG2, sites less likely to be directly involved in disease pathogenesis. Conversely, others reported that IgE autoantibodies in BP patients target the NC16A region of BPAG2. It has been suggested that such anti-BPAG2 IgE autoantibodies may bind to IgE receptors on the surface of dermal mast cells and basophils. Subsequent binding of BPAG2 fragments (such as the 120-kD processed ectodomain) to the mast cell-bound IgE may then result in activation and degranulation of mast cells, setting off an inflammatory cascade. Interestingly, a recent study showed that a recombinant NC16A fusion protein degranulated basophils opsonized with IgE from patients with BP. These findings support the hypothesis that anti-BPAG2 IgE autoantibodies contribute to the pathogenesis of BP by degranulation of dermal mast cells [13].

Animal Models of BP

Early attempts to develop an animal model of BP generally failed to result in inflammatory skin lesions. Interestingly, rabbits immunized with an 18-amino acid peptide corresponding to a portion of BPAG1 recognized by circulating autoantibodies from patients with BP developed an enhanced inflammatory reaction to UVB irradiation. Light microscopy studies of the irradiated skin revealed an inflammatory infiltrate of neutrophils at the dermal-epidermal junction and linear deposits of IgG and C3 in epidermal BM. These findings demonstrated that antibodies against a BPAG1 synthetic peptide can lead to enhanced inflammatory responses after epithelial injury in rabbit skin. In a recent study, polyclonal antibodies were generated against an antigenic sequence of BPAG1 (specifically, amino acids 2479–2499) that shows 67% homology between mouse and man [14]. Passive transfer of this purified anti-BPAG1 IgG into neonatal isogeneic CBA/Ca mice induced subepidermal blisters. Immunohistological examinations revealed linear deposits of rabbit IgG and mouse C3 in the epidermal BM of perilesional skin. An intradermal inflammatory reaction of granulocytes was also detected. These findings show that antibodies against BPAG1 can elicit the clinical and immunopathological features of BP in neonatal mice, suggesting that anti-BPAG1 autoantibodies may play a pathogenic role in this disease.

Passive transfer of experimental IgG developed against the murine homolog of the immunodominant portion of the BPAG2 NC16A domain into BALB/c mice produced clinical, histologic and immunopathologic alterations like those seen in patients with BP [15]. Antibody-induced blister formation in this animal model is dependent upon the activation of complement, degranulation of dermal mast cells and generation of neutrophil-rich infiltrates. The finding that matrix metalloproteinase-9-deficient mice are resistant to anti-NC16A IgG has been explained by the observation that neutrophil-derived matrix metalloproteinase-9 inactivates 1-proteinase inhibitor, which allows unrestrained activity of neutrophil elastase that degrades BPAG2 and produces subepidermal blisters in this experimental model. Studies have shown that this matrix metalloproteinase-9 activation is plasmin dependent, and that plasmin activates matrix metalloproteinase-9 independent of matrix metalloproteinase-3.

In other studies, a keratin 14 promoter construct was used to produce transgenic (Tg) mice expressing human BPAG2 in murine epidermal BM [16]. Grafts of Tg skin placed on gender-matched, syngeneic wild-type or MHC I^{−/−} mice elicited IgG that bound human epidermal BM and BPAG2. Tg grafts on wild-type mice developed granulocyte-rich infiltrates, dermal edema, subepidermal blisters and deposits of immunoreactants in epidermal BM. This model shows fidelity to alterations seen in patients with BP, has relevance to

immune responses that may arise in patients with EB following BPAG2 gene replacement, and can be used to identify interventions that may block unwanted production of IgG against proteins in epidermal BM. In related studies, these same human BPAG2 Tg mice were used to rescue the blistering phenotype of mice in which the gene encoding *COL17A1* had been inactivated (that is, BPAG2^{-/-} mice) [17]. Interestingly, passive transfer of IgG from patients with BP into these BPAG2-humanized mice induced subepidermal blisters that displayed clinical, histological and immunopathologic features of BP. Injection of short, recombinant decoy peptides (including peptides corresponding to the immunodominant NC16A domain) significantly suppressed blister formation by binding and neutralizing pathogenic IgG from patients with BP in this model.

Precipitating Factors

Although most cases of BP occur sporadically without any obvious precipitating factors, several reports have suggested that UV (specifically, UVB or PUVA) or ionizing irradiation may induce (or provoke) BP. Interestingly, several case reports have suggested that infestation with scabies may also induce BP. Several medications have been implicated in precipitating a clinically heterogeneous group of bullous disorders with similarities to BP. The majority of these drugs contain free sulfhydryl groups, either within the moiety of the parent compound or within a metabolite. It has been proposed that the thiol group may allow the molecule to combine with proteins in the lamina lucida, act as a hapten, and result in autoantibody formation to BM proteins. On the other hand, other drugs are thought to cause subepidermal blistering independent of an immune response. Many of the implicated pharmacological agents (for example, vaccines or treatments such as electron beam therapy) have only been temporally associated with the onset of BP-like lesions in middle-aged to elderly individuals who are susceptible to develop BP. Temporal occurrence in younger patients where BP is extremely rare is more supportive of a causative association. Some medications (such as penicillamine) have been cited repeatedly for eliciting reactions that resemble pemphigus or pemphigoid. Drugs such as furosemide, phenacetin, enalapril, ibuprofen, amoxicillin, ampicillin, penicillin, β -blockers and influenza vaccinations have also been implicated for inducing BP (and in some cases, indeed verified by rechallenge). Spontaneous resolution of BP after drug withdrawal has been described in patients on spironolactone, bumetanide and fluoxetine. Drug-induced BP can develop 1–90 days following consumption of the offending agent. While drug-induced BP can mirror the clinical findings of native disease, many reports suggest more severe (but nonscarring) mucosal and/or acral (specifically palm or sole) involvement in such cases.

Differential Diagnosis

BP can usually be differentiated from LAD, chronic bullous disease of childhood (CBDC), dermatitis herpetiformis (DH), erythema multiforme and pemphigus vulgaris by light and IF microscopy studies. In contrast to BP, lesions in patients with pemphigus vulgaris form as a consequence of acantholysis and show deposits of IgG on the surface of keratinocytes. EBA and MMP may be more challenging to differentiate from BP. EBA is often characterized by skin fragility, trauma-induced lesions, absence of inflammation, healing with milia and scarring, and lesions localized to areas exposed to pressure or trauma. By light microscopy, there may be a subepidermal blister with a paucicellular inflammatory base in patients with EBA. However, EBA may occasionally present as an inflammatory disease with clinical, histological and routine direct IF studies like those seen in patients with BP. Direct IF microscopy studies of perilesional skin from patients with EBA show linear, u-shaped deposits of IgG in serrated portions of epidermal BM, in contrast to n-shaped deposits of IgG in serrated portions of epidermal BM from patients with BP. Autoantibodies from patients with EBA are directed against type VII collagen in anchoring fibrils. Thus, indirect IF microscopy studies of sera from patients with EBA bind the dermal side of 1 M NaCl split skin. In contrast to BP, MMP presents predominantly, if not exclusively, on mucous membranes. If there is blistering of the skin in patients with MMP, it is usually transient or of limited extent. Large, tense blisters, which are characteristic of BP, are usually not seen in patients with MMP. Direct IF microscopy of normal-appearing perilesional tissue from patients with MMP shows continuous deposits of immunoreactants (most commonly IgG and C3) in epithelial BMs. Indirect IF microscopy studies of sera from patients with MMP typically show IgG (and/or IgA) directed against the epidermal side of 1 M NaCl split skin; however, combined epidermal and dermal staining, or exclusively dermal binding can occur in some patients. Some forms of LAD clinically resemble BP or DH. However, these patients are distinguished by continuous deposits of IgA in their epidermal BMs and (in many cases) by circulating IgA anti-BM autoantibodies.

Treatment and Prognosis

BP is generally regarded as a self-limited disease of approximately 6–10 years duration. Exacerbations and remissions are common. Morbidity in patients with BP can be considerable. The mortality rate in various studies ranges from 11 to 40% at 1 year. For the most part, treatment of patients with autoimmune blistering diseases is grounded in clinical experience rather than randomized controlled clinical trials. BP of minimal severity and/or distribution can sometimes be controlled with topical glucocorticoids. However, the mainstay of treatment for this disorder is systemic glucocorticoids, with initial daily

morning doses of prednisone typically in the range of 0.75–1.0 mg/kg per day. Patients with extensive disease may require the addition of other immunosuppressive agents (for example azathioprine, mycophenolate mofetil or cyclophosphamide) to a regimen of daily glucocorticoids. Dapsone, antibiotics, intravenous immunoglobulin or biologicals may be of benefit in selected patients. Such treatments have reduced the morbidity and mortality of BP. Unfortunately, both still occur as a consequence of this disease and/or its treatment in elderly and/or debilitated patients. Measures to minimize the adverse effects of chronic treatment with systemic glucocorticoids are warranted. Rituximab, an anti-CD20 humanized monoclonal antibody leading to transitory B cell depletion, has recently been used successfully for recalcitrant cases.

Mucous Membrane Pemphigoid

Epidemiology

MMP is a rare autoimmune subepidermal blistering disease characterized by erosive lesions of mucous membranes and skin that results in scarring in at least some sites of involvement [18]. Lesions commonly involve the oral mucosa and the conjunctivae. Other sites that may be affected include the nasopharyngeal, laryngeal, esophageal and anogenital mucosae. Skin lesions are present in about one third of patients, tend to predominate on the scalp, face and upper trunk, and usually consist of a few scattered erosions or tense blisters on an erythematous or urticarial base. MMP is typically chronic and progressive; it may result in serious complications.

MMP is rare, occurring in perhaps 1 person per million annually. Females are affected 1.5–2 times as often as males. MMP is a disease with a mean age of onset in the early to middle 60s. Although there is no racial or geographic predilection, some forms have been associated with certain immunogenetic haplotypes. Specifically, ocular MMP has been associated with HLA-DR4 and DQw7, while HLA-DQB1*0301 has been associated with both oral and ocular forms of this disease.

Clinical Features

The mouth is the most frequent site of involvement in patients with MMP; it is often the first and only site affected. Lesions in the mouth often involve the gingiva, buccal mucosa and palate; other sites such as the alveolar ridge, tongue and lips are also susceptible. The most frequent oral manifestation is desquamative gingivitis. Other lesions may present as tense blisters that rupture easily or as mucosal erosions that form as a consequence of epithelial fragility. Lesions in the mouth may result in a delicate white pattern of reticulated scarring.

Gingival involvement can result in tissue loss and dental complications (for example caries, periodontal ligament damage, as well as loss of bone mass and teeth). Ocular involvement is common and may be sight threatening. Ocular lesions typically manifest as conjunctivitis that progresses insidiously to scarring. MMP-induced ocular scarring is characterized by shortened fornices, symblephara and, in severe disease, ankyloblephara. Conjunctival scarring can also cause entropion and trichiasis resulting in corneal irritation, superficial punctate keratinopathy, corneal neovascularization, ulceration and blindness. Slit-lamp examination by an ophthalmologist is crucial to detect early disease, as it may be subtle, but can progress to severe complications. MMP may be limited to the eyes.

The skin is involved in 25–35% of patients with MMP, most frequently on the scalp, head, neck and upper trunk. Lesions typically consist of small vesicles or bullae situated on erythematous and/or urticarial bases. Lesions rupture easily and are often seen as small, crusted papules or plaques. In general, the extent and number of cutaneous lesions are small. Other mucous membranes that may be involved in MMP include the nasopharyngeal, laryngeal, esophageal and anogenital mucosae.

Pathology

Light microscopy studies of lesional skin or mucosa from patients with MMP show subepidermal blisters and a mixed leukocytic infiltrate. Mononuclear cells, histiocytes and plasma cells dominate in lesional sites on mucosa; eosinophils and neutrophils are more commonly seen in skin lesions. Biopsies of older lesions may be relatively cell poor and correlate with the non-inflammatory character of such sites clinically. Older lesions may also display fibrosis in the papillary dermis. Electron microscopy studies have shown that blisters of skin and mucous membranes in patients with MMP develop within the lamina lucida. It is a generally held view that blisters in MMP form below those seen in patients with BP, since scarring is more common in this disease.

Immunopathology

Direct IF microscopy of normal-appearing perilesional tissue from patients with MMP shows continuous deposits of immunoreactants (most commonly IgG and/or C3) in epithelial BMs. IgA, IgM and/or fibrin are found in the epithelial BMs of some patients. Indirect IF microscopy studies using 1 M NaCl split skin show IgG (and/or IgA) directed usually to the epidermal side, although combined epidermal and dermal, or exclusively dermal binding can occur in some patients. In fact, this heterogeneity in autoantibody binding patterns was one of the first clues that MMP is associated with autoantibodies of different specificity.

Circulating anti-BM autoantibodies of low to moderate titer are present in many MMP patients. Over the last 15 years, studies in a number of different laboratories have shown that: (1) autoantibodies from patients with MMP target different autoantigens in epithelial BMs; (2) autoantibodies in a given patient usually retain their specificity (that is, they target the same autoantigen throughout the course of the disease); (3) several MMP autoantigens represent important adhesion proteins in epidermal BM. Therefore, MMP is currently thought to be not a single disease, but rather a phenotype that develops as a consequence of autoantibody-mediated tissue damage to epithelial BMs. Many MMP patients who have circulating IgG that binds to the epidermal side of 1 M NaCl split skin have autoantibodies reactive with BPAG2 [19]. Autoantibodies in these patients typically bind the distal extracellular domain of this transmembrane molecule as well as its NC16A domain. Interestingly, immunoelectron microscopy studies have shown that anti-BPAG2 IgG from patients with MMP localizes within the lower part of the lamina lucida near its junction with the lamina densa, a site that corresponds to the localization of the distal extracellular domain of BPAG2 in epidermal BM. This observation may explain the greater likelihood of scar formation in patients with this disease rather than those with BP and PG.

In contrast to the patients described above, some MMP patients have IgG autoantibodies directed exclusively against the dermal side of 1 M NaCl split skin [20]. Some of these patients have autoantibodies that bind the superior surface of the lamina densa at its interface with the lamina lucida. Molecular studies have shown that these patients' IgG anti-BM autoantibodies are directed against a set of disulfide-linked polypeptides produced by human keratinocytes. Because the protein that was initially identified as this autoantigen was at the time termed epiligrin, this form of disease was called antiepiligrin cicatricial pemphigoid. Subsequent studies showed that epiligrin is a laminin isoform, namely laminin 5 ($\alpha 3\beta 3\gamma 2$), and that most of these patients have autoantibodies that bind the α subunit of this protein [21]. Because this subunit is also utilized in laminin 6 ($\alpha 3\beta 1\gamma 1$), these patients' IgG recognize this protein as well. Another subgroup of MMP patients who have IgG directed against the dermal side of 1 M NaCl split skin have autoantibodies directed against type VII collagen in anchoring fibrils. These patients represent a mucosal-predominant form of EBA that is generally indistinguishable from MMP.

Pathophysiology and Animal Models

As noted earlier, BPAG2 is the autoantigen most commonly recognized by autoantibodies from MMP patients who have IgG that binds the epidermal side of 1 M NaCl split skin. While passive transfer studies have demonstrated that experimental IgG directed against the NC16A domain of BPAG2 is pathogenic

in neonatal mice, to date there is no evidence that antibodies directed against the distal extracellular domain of BPAG2 are pathogenic in experimental animals or humans. In contrast, passive transfer of anti-laminin 5 IgG to neonatal or adult mice induces subepidermal blisters of skin and mucous membranes that display clinical, histologic and immunopathologic features like those seen in patients with antiepiligrin cicatricial pemphigoid [22]. Anti-laminin 5 IgG induces the same lesions in complement-, mast cell- or T cell-deficient mice, suggesting that such antibodies may elicit epidermal detachment in vivo in a noninflammatory and direct manner. Studies have also shown that injection of IgG from patients with this form of MMP into human skin grafts on immunodeficient mice elicits noninflammatory subepidermal blisters, confirming that these patients' autoantibodies are pathogenic in vivo.

Systemic Associations

A cohort of patients with antiepiligrin cicatricial pemphigoid was reported to have an increased relative risk for solid cancer. This form of cicatricial pemphigoid appears to have a relative risk for malignancy that approximates that for adults with dermatomyositis; the risk for cancer appears to be highest in the first year of disease.

Brunsting-Perry Pemphigoid

In 1957, Brunsting and Perry described seven patients with locally recurrent, scarring subepidermal blistering lesions on the head or neck that for many years were considered to be a form of MMP. This variant form of disease was said to predominate in men and to lack mucous membrane involvement. Recently, most patients with these clinical, histologic and immunopathologic features have been reported to have autoantibodies against type VII collagen (therefore representing a localized form of EBA). Less commonly, such patients have autoantibodies that bind BPAG1 and/or BPAG2.

Treatment and Prognosis

MMP is typically a chronic and progressive disorder, though involvement may be limited to a given anatomic site for many years. MMP rarely goes into spontaneous remission; its treatment is largely tempered by its severity and sites of involvement. Scarring can only be prevented but not reversed. Mild lesions of the oral mucosa and skin can sometimes be effectively treated with topical glucocorticoids. For oral disease resistant to topical glucocorticoids, these agents can be administered intralesionally. Use of dapsone for treatment of MMP is somewhat controversial. In severe cases, systemic glucocorticoids can be administered alone (for example 20–40 mg of prednisone orally each morning) or in combination with dapsone. Because of potentially severe complications,

ocular, laryngeal, esophageal and/or anogenital involvement requires aggressive management by a multidisciplinary team. For resistant and severe ocular disease, a combination of systemic glucocorticoids and an immunosuppressive agent is often indicated. In such cases, cyclophosphamide (1–2 mg/kg per day) is usually tried in conjunction with daily prednisone first. Once remission is achieved, these medications can be reduced and, in patients free of recurring disease, stopped. All patients require long-term follow-up due to the potential of this disease to relapse. Recent case reports described successful treatment of severe, treatment-resistant MMP with TNF- α inhibitors (specifically, etanercept and infliximab).

Pemphigoid Gestationis

PG is a rare subepidermal bullous disease of pregnancy and puerperium. Its prior designation as herpes gestationis (HG) relates to the grouped character of lesions typically seen in these patients; the disorder has no relationship to an existing or prior viral infection.

Epidemiology

PG is rare; estimates suggest that the incidence of this disorder in pregnancies in Caucasians in North America is about 1 to 50,000. PG is less common in African Americans. Patients with PG commonly display HLA-B8 and the HLA-DR3/DR4 paired haplotypes. Complement polymorphism studies demonstrated a 90% frequency of the C4 null allele in patients with PG.

Clinical Features

PG may begin during any trimester of pregnancy or just after delivery; patients previously affected tend to experience earlier onset of disease in subsequent gestations [23]. Lesions typically involve the abdomen (especially within the umbilicus), trunk and extremities; mucous membrane and facial lesions are uncommon. Like those seen in patients with BP, lesions in PG are quite polymorphic and may range from urticarial papules and plaques to vesicles or frank tense bullae. Lesions in PG are almost always pruritic. Exacerbations of PG often occur immediately after delivery; brief flares of disease may develop with resumption of menses or on exposure to oral contraceptives in previously affected patients. Although PG was once thought to be linked to an increased incidence of fetal morbidity and mortality, there is now agreement that neonates of affected women are only at increased risk of being premature. The hormonal (or other) influences responsible for the development of PG remain to be determined, although its novel link to the gestational state is substantiated by rare

cases that have developed in patients with underlying choriocarcinoma or hydatidiform mole.

Pathology

Biopsies of early lesional skin from patients with PG typically show teardrop-shaped subepidermal blisters in association with an eosinophil-rich leukocytic infiltrate – findings virtually indistinguishable from those seen in patients with BP. There is a superficial perivascular infiltrate (of varying intensity) composed of lymphocytes, histiocytes, eosinophils and occasionally a few neutrophils. The presence of eosinophils is the most constant histologic feature of PG. There is edema of the dermal papillae and epidermis, associated with characteristic foci of basal cell necrosis over tips of dermal papillae. Although these findings are characteristic, they are not diagnostic of PG.

Immunopathology

Direct IF microscopy of perilesional skin from patients with PG demonstrates linear deposits of C3 in epidermal BM, the immunopathologic hallmark of this subepidermal blistering disease [24]. In addition to C3, 30–50% of these patients also demonstrate linear deposits of IgG in epidermal BM. Other immunoreactants (for example IgA, IgM, C1q and C4) can also be found within the epidermal BM of selected patients. Immunoreactants are deposited in the lamina lucida, the same ultrastructural subregion of epidermal BM in which blister formation occurs. Immunoreactants persist in situ in the skin of patients with PG for as long as 6–18 months after the eruption is controlled by treatment or resolves postpartum. Immunoreactants are found both in lesional as well as nonlesional skin of patients with PG. Direct IF microscopy of skin from infants of affected mothers reveals the same pattern and character of immunoreactants in epidermal BM. About 25% of patients with PG demonstrate circulating anti-BM IgG autoantibodies in routine indirect IF microscopy studies using normal human skin as a test substrate; a greater percentage of patients shows autoantibodies reactive with epidermal BM when 1 M NaCl split skin is used as a test substrate. In contrast to routine indirect IF testing, complement fixation indirect IF microscopy studies demonstrate that most patients with PG possess low-titer, circulating anti-BM IgG.

Pathophysiology

Historically, the entity in the sera of patients with PG that is responsible for its reactivity with epidermal BM as well as its complement fixing ability was designated the HG factor. The HG factor now is recognized as a low-titer IgG anti-BM autoantibody that displays avid complement fixing ability. It has been hypothesized that IgG autoantibodies in patients with PG are initiated in

response to an antigenic stimulus peculiar to pregnancy – one perhaps residing in the amnion. The demonstration that autoantibodies from patients with PG recognize the same autoantigen bound by IgG from patients with BP confirmed on a molecular level the similarity of the clinical, histologic and immunologic features of these two autoimmune diseases. The pathogenic nature of autoantibodies in patients with PG was substantiated not only by experimental studies, but also by the development of transient skin lesions in infants as a consequence of transplacental passage. The vast majority of BP and PG patients has circulating IgG reactive with the membrane-proximal NC16A domain of BPAG2. Interestingly, some studies indicate that the anti-BPAG2 autoantibody reactivity profile is different between PG and BP. More specifically, BP patients have autoantibodies that bind several BPAG2 epitopes other than NC16A, whereas the autoantibody reactivity in PG patients appears to be mostly restricted to NC16A. Both IgG₁ and IgG₃ subclasses of autoantibodies have been noted in PG patients. In addition, anti-BM autoantibodies in some patients recognize BPAG1. The pathogenic relevance of anti-BPAG2 autoantibodies in patients with PG is supported by cases where transplacental passage of anti-BPAG2 IgG causes transient blistering in neonates.

Interestingly, autoantibodies from patients with PG bind amniotic BM, a structure derived from fetal ectoderm that is antigenically similar to skin. Women with PG also show an increased expression of MHC class II antigens (for example HLA-DR, HLA-DP and HLA-DQ) within the villous stroma of chorionic villi. It has therefore been proposed that PG is a disease initiated by the aberrant expression of MHC class II determinants (perhaps of paternal origin) that serve to initiate an allogeneic response to placental BM, which in turn cross-reacts with skin. This theory is supported by the finding that the incidence of anti-HLA antibodies is essentially 100% in patients with a history of PG.

Treatment and Prognosis

Most patients with PG require treatment with moderate (or higher) doses of daily glucocorticoids (for example prednisone 0.5–1.0 mg/kg per day) at some point in the course of their disease. Despite concern about the use of such medications during gestation, current evidence suggests that there is no difference in the frequency of uncomplicated live births in patients with PG treated with systemic glucocorticoids versus those treated with more conservative measures. Postpartum flares of PG may require interim treatment with high doses of prednisone (for example 1.0 mg/kg per day) even in patients whose disease was previously controlled or in remission. If systemic glucocorticoids are administered during pregnancy, newborns are at risk for development of reversible adrenal insufficiency. Cutaneous lesions noted in affected infants are of a transient nature and require no specialized therapy.

Linear IgA Dermatitis

LAD is an autoimmune subepidermal blistering disease characterized by circulating IgA anti-BM autoantibodies [25]. Patients with LAD can display a phenotype like that seen in patients with BP, MMP, EBA or DH. CBDC is a rare subepidermal blistering disease that occurs predominantly in children younger than 5 years and, like LAD, is characterized by circulating IgA anti-BM autoantibodies [26].

Epidemiology

LAD most often occurs after the fourth decade of life; there is a slight predominance in females. Both LAD and CBDC are associated with the HLA-B8 haplotype.

Clinical Features

The clinical features of LAD, like those of BP, include tense vesicles and bullae that develop de novo or on an inflamed, urticarial base. Alternatively, LAD may resemble DH as a pruritic papulovesicular eruption involving extensor surfaces symmetrically. Bullae in LAD may be somewhat linear or sausage shaped. The torso and limbs are most frequently involved, though involvement of the hands, feet, perineum and face may also be seen. Mucous membrane involvement has been reported to occur in up to 80% of patients. In CBCD, blisters are frequently located on the lower abdomen and perineum and have a configuration known as ‘cluster of jewels’ where new lesions occur at the periphery of older blisters. The torso, hands, feet and face may also be affected. Mucosal involvement in CBCD has been reported to occur in up to two thirds of patients. Occasionally, LAD may develop as an atypical eruption resembling erythema multiforme.

Pathology

Lesional skin from patients with LAD shows subepidermal bulla with a superficial dermal infiltrate of neutrophils. Papillary microabscesses composed of neutrophils, similar to those seen in DH, may occur, though more commonly neutrophils tend to be scattered evenly along the BM in lesional skin from patients with LAD. Rarely, eosinophils may be admixed among the neutrophilic infiltrate. It is often difficult to distinguish LAD from DH or the bullous eruption of systemic lupus erythematosus by histology alone.

Immunopathology

Direct IF microscopy of perilesional skin shows linear deposits of IgA in epidermal BM. Cases of LAD with linear deposits of IgG in addition to IgA

deposited in epidermal BM have been reported. Anti-BM autoantibodies in patients with LAD are usually of the IgA₁ subclass, though IgA₂ autoantibodies have been described in rare cases. Circulating IgA anti-BM autoantibodies are often detected in the sera of patients with LAD; most patients have anti-BM autoantibodies that bind the epidermal side of 1 M NaCl split skin. Rare patients have IgA autoantibodies that bind both epidermal and dermal sides of 1 M NaCl split skin. Immunoblot studies showed that circulating autoantibodies from most patients with LAD bind a 120-kD protein in the conditioned medium of cultured human keratinocytes termed LAD-1. Biochemical studies have shown that LAD-1 (and a smaller 97-kD fragment termed LAD97) are generated by proteolytic cleavage of the BPAG2 ectodomain as discussed earlier.

Pathophysiology

LAD may be either idiopathic or, in a minority of cases, drug induced. The mechanism for blister formation is not fully understood, but is likely to involve IgA- and complement-mediated neutrophil chemotaxis. Passive transfer of IgA murine monoclonal antibodies against the LAD autoantigen to SCID mice bearing human skin grafts produced neutrophil-rich infiltrates and subepidermal vesicles in some of the mice challenged [27]. While multiple drugs have been reported to cause LAD, vancomycin is the most common culprit. The mechanism by which these drugs stimulate pathogenic IgA anti-BM autoantibodies is unknown. In many cases, the eruption has remitted with discontinuation of the drug and recurred with its reintroduction. Interestingly, LAD has been reported in association with both lymphoid and nonlymphoid malignancies.

Differential Diagnosis

Clinical findings in patients with LAD can mimic DH, BP, MMP or EBA. Patients with CBDC must be differentiated from those with childhood DH or childhood BP. Linear immunodeposits of IgA, most often in the absence of IgG and C3, can distinguish LAD from these other immunobullous diseases.

Treatment and Prognosis

Adults with LAD have an unpredictable course. Many patients have a chronic disease that continues for years, with few if any episodes of remission. Alternatively, patients may have a spontaneous remission and disappearance of linear IgA deposits in epidermal BM. Patients with severe mucosal disease, especially of the eyes, may develop notable scarring.

Patients with LAD typically respond to dapsone or sulfapyridine. This response usually occurs within 24–48 h. If dapsone or sulfapyridine fail to adequately control disease, daily low doses of prednisone (for example 5–10 mg per day) may be helpful. Other therapies that have been reported to be of benefit

in LAD include colchicine and combinations of tetracycline and niacinamide. Rare patients require treatment with systemic glucocorticoids and additional immunosuppressives such as azathioprine or mycophenolate mofetil. CBDC is usually a self-limited disease, with many children going into remission within 2 years. Occasionally, the disease persists into puberty. As in LAD, the treatment of choice for patients with CBDC is dapsone or sulfapyridine. Small daily doses of prednisone may be required to bring CBDC under control.

Lichen Planus Pemphigoides

The term ‘lichen ruber pemphigoides’ or lichen LPP was first used by Kaposi in 1892 for a disease characterized by lesions of lichen planus (LP) and numerous bullae [28]. LPP is characterized by tense blisters atop and/or apart from lesions of LP. In bullous LP, blisters are confined to LP lesions and are a consequence of severe basal cell degeneration. Conversely, in LPP, light microscopy studies identify typical features of LP in papular lesions and changes of BP in biopsies of blistered skin. Most importantly, the finding of linear deposits of IgG and/or C3 in epidermal BM of perilesional skin differentiates LPP from bullous LP. Circulating IgG autoantibodies in patients with LPP may target different antigens, including BPAG1, BPAG2 as well as a yet uncharacterized 200-kD epidermal protein. Like sera from most BP patients, LPP sera react with the immunodominant NC16A domain of BPAG2. Within the NC16A domain, LPP sera recognize an epitope located at its carboxyl terminus. Several observations speak against the association of LP and BP in LPP patients. For example, LPP is seen in younger patients (the reported average age is 44 years) compared with BP; also, in contrast to BP, blisters in patients with LPP are preferentially distributed over distal extremities. The course of LPP is variable and lesions may either abate spontaneously or remit with systemic glucocorticoids, azathioprine, dapsone or a combination of various agents.

Conclusion

BPAG2 is a type II transmembrane collagen associated with HDs in basal keratinocytes. The BPAG2 ectodomain consists of 15 interrupted collagen domains; its largest noncollagenous domain, NC16A, is located adjacent to plasma membranes of basal keratinocytes. Five autoimmune subepidermal blistering diseases are known to be associated with an autoimmune response to BPAG2, namely BP, MMP, PG, LAD and LPP. These diseases demonstrate that different clinical phenotypes are associated with an autoimmune response to

the same skin autoantigen. Animal models of these diseases and advances in translational research have the potential to elucidate the pathophysiology of these disorders and identify new approaches for their treatment.

References

- 1 Yancey KB: The pathophysiology of autoimmune blistering diseases. *J Clin Invest* 2005;115:825–828.
- 2 Yancey KB, Egan CA: Pemphigoid: clinical, histologic, immunopathologic, and therapeutic considerations. *JAMA* 2000;284:350–356.
- 3 Masunaga T: Epidermal basement membrane: its molecular organization and blistering disorders. *Connect Tissue Res* 2006;47:55–66.
- 4 Borradori L, Sonnenberg A: Structure and function of hemidesmosomes: more than simple adhesion complexes. *J Invest Dermatol* 1999;112:411–418.
- 5 Stanley JR, Hawley-Nelson P, Yuspa SH, Shevach EM, Katz SI: Characterization of bullous pemphigoid antigen: a unique basement membrane protein of stratified squamous epithelia. *Cell* 1981;24:897–903.
- 6 Labib RA, Anhalt GJ, Patel HP, Mutsim DF, Diaz LA: Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. *J Immunol* 1986;136:1231–1235.
- 7 Diaz LA, Rattie H 3rd, Saunders WS, Futamura S, Squiquera HL, Anhalt GJ, Giudice GJ: Isolation of a human epidermal cDNA corresponding to the 180-kD autoantigen recognized by bullous pemphigoid and herpes gestationis sera. Immunolocalization of this protein to the hemidesmosome. *J Clin Invest* 1990;86:1088–1094.
- 8 Van den Bergh F, Giudice GJ: BP180 (type XVII collagen) and its role in cutaneous biology and disease. *Adv Dermatol* 2003;19:37–71.
- 9 Zillikens D, Giudice GJ: BP180/type XVII collagen: its role in acquired and inherited disorders or the dermal-epidermal junction. *Arch Dermatol Res* 1999;291:187–194.
- 10 Hall RP 3rd, Murray JC, McCord MM, Rico MJ, Streilein RD: Rabbits immunized with a peptide encoded for by the 230-kD bullous pemphigoid antigen cDNA develop an enhanced inflammatory response to UVB irradiation: a potential animal model for bullous pemphigoid. *J Invest Dermatol* 1993;101:9–14.
- 11 Zillikens D, Rose PA, Balding SD, Liu Z, Olague-Marchan M, Diaz LA, Giudice GJ: Tight clustering of extracellular BP180 epitopes recognized by bullous pemphigoid autoantibodies. *J Invest Dermatol* 1997;109:573–579.
- 12 Hertl M, Eming R, Veldman C: T cell control in autoimmune bullous skin disorders. *J Clin Invest* 2006;116:1159–1166.
- 13 Fairley JA, Fu CL, Giudice GJ: Mapping the binding sites of anti-BP180 immunoglobulin E autoantibodies in bullous pemphigoid. *J Invest Dermatol* 2005;125:467–472.
- 14 Kiss M, Husz S, Janossy T, Marczinovits I, Molnar J, Korom I, Dobozy A: Experimental bullous pemphigoid generated in mice with an antigenic epitope of the human hemidesmosomal protein BP230. *J Autoimmun* 2005;24:1–10.
- 15 Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, Giudice GJ: A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. *J Clin Invest* 1993;92:2480–2488.
- 16 Olasz EB, Roh J, Yee CL, Arita K, Akiyama M, Shimizu H, Vogel JC, Yancey KB: Human bullous pemphigoid antigen 2 transgenic skin elicits specific IgG in wild-type mice. *J Invest Dermatol* 2007;127:2807–2817.
- 17 Nishie W, Sawamura D, Goto M, Ito K, Shibaki A, McMillan JR, Sakai K, Nakamura H, Olasz E, Yancey KB, Akiyama M, Shimizu H: Humanization of autoantigen. *Nat Med* 2007;13:378–383.
- 18 Yancey KB: Cicatricial pemphigoid; in Freedburg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, Fitzpatrick TB (eds): *Fitzpatrick's Dermatology in General Medicine*, ed 5. New York, McGraw-Hill, 2002, pp 674–680.

- 19 Bedane C, McMillan JR, Balding SD, Bernard P, Prost C, Bonnetblanc JM, Diaz LA, Eady RA, Giudice GJ: Bullous pemphigoid and cicatricial pemphigoid autoantibodies react with ultrastructurally separable epitopes on the BP180 ectodomain: evidence that BP180 spans the lamina lucida. *J Invest Dermatol* 1997;108:901–907.
- 20 Domloge-Hultsch N, Gammon WR, Briggaman RA, Gil SG, Carter WG, Yancey KB: Epiligrin, the major human keratinocyte integrin ligand, is a target in both an acquired autoimmune and an inherited subepidermal blistering skin disease. *J Clin Invest* 1992;90:1628–1633.
- 21 Kirtschig G, Marinkovich MP, Burgeson RE, Yancey KB: Anti-basement membrane autoantibodies in patients with anti-epiligrin cicatricial pemphigoid bind the alpha subunit of laminin 5. *J Invest Dermatol* 1995;105:543–548.
- 22 Lazarova Z, Yee C, Darling T, Briggaman RA, Yancey KB: Passive transfer of anti-laminin 5 antibodies induces subepidermal blisters in neonatal mice. *J Clin Invest* 1996;98:1509–1518.
- 23 Jenkins RE, Hern S, Black MM: Clinical features and management of 87 patients with pemphigoid gestationis. *Clin Exp Dermatol* 1999;24:255–259.
- 24 Katz SI, Hertz KC, Yaoita H: Herpes gestationis. Immunopathology and characterization of the HG factor. *J Clin Invest* 1976;57:1434–1441.
- 25 Egan CA, Zone JJ: Linear IgA bullous dermatosis. *Int J Dermatol* 1999;38:818–827.
- 26 Wojnarowska F: Chronic bullous disease of childhood. *Semin Dermatol* 1988;7:58–65.
- 27 Zone JJ, Egan CA, Taylor TB, Meyer LJ: IgA autoimmune disorders: development of a passive transfer mouse model. *J Invest Dermatol Symp Proc* 2004;9:47–51.
- 28 Harting MS, Hsu S: Lichen planus pemphigoides: a case report and review of the literature. *Dermatol Online J* 2006;12:10.

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Pemphigus Vulgaris and Its Active Disease Mouse Model

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Abstract

Pemphigus is an autoimmune disease of the skin and mucous membranes and is mediated by IgG autoantibodies against desmoglein (Dsg), a cadherin-type cell-cell adhesion molecule in desmosomes. Recently, an active disease mouse model of pemphigus vulgaris (PV) was generated with a unique approach using autoantigen knockout mice, in which self-tolerance of the defective gene product is not acquired. This approach included the adoptive transfer of Dsg3^{-/-} lymphocytes to Rag2^{-/-} immunodeficient mice that express Dsg3-induced stable production of pathogenic anti-Dsg3 IgG for over 6 months and the phenotype of PV including oral erosion with the typical histology in recipient mice. Subsequently, AK and NAK series of anti-Dsg3 IgG monoclonal antibodies were developed from the PV model mice. These monoclonal antibodies showed pathogenic heterogeneity in blister formation, which is, at least in part, explained by their epitopes, and synergistic pathogenic effects by combining several monoclonal antibodies reacting in different parts of the molecule. Although this model does not reflect the actual triggers of autoimmune diseases, it does provide a means to investigate the roles of T and B lymphocytes in perpetuating autoantibody production and to clarify unsolved immunological mechanisms in the autoimmune diseases.

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Pemphigus as an Autoimmune Disease against Desmosomal Cadherin

Pemphigus is a group of chronic blistering skin diseases in which autoantibodies are directed against the cell surface of keratinocytes, resulting in the loss of cell-cell adhesion of keratinocytes through a process called acantholysis [1]. Pemphigus can be divided into three major forms: pemphigus vulgaris (PV), pemphigus foliaceus (PF) and paraneoplastic pemphigus.

In PV, essentially all patients have mucosal membrane erosions and more than half of them also have skin blisters and erosions. The blisters of PV develop in the deeper part of the epidermis, just above the basal cell layer. In PF, patients have only cutaneous involvement without mucosal lesions and the splits occur in the superficial part of the epidermis, mostly at the granular layer. Paraneoplastic pemphigus has more recently been recognized as a disease distinct from the classic forms of pemphigus. Patients with paraneoplastic pemphigus have a known or occult associated neoplasm, usually of lymphoid tissue. Painful severe oral and conjunctival erosions are a prominent feature of paraneoplastic pemphigus.

The hallmark of pemphigus is the finding of IgG autoantibodies against the cell surface of keratinocytes [2]. The pemphigus autoantibodies found in patients' sera play a primary pathogenic role in inducing the loss of cell adhesion of keratinocytes with resultant blister formation. Immunochemical characterization of pemphigus antigens by immunoprecipitation or immunoblotting with extracts from cultured keratinocytes or epidermis has demonstrated that the PV and PF antigens are 130-kDa and 160-kDa transmembrane glycoproteins, respectively [3]. Molecular cloning of cDNA encoding pemphigus antigens indicates that both these molecules are members of the cadherin supergene family [4, 5]. PF and PV antigens are termed desmoglein 1 (Dsg1) and Dsg3, respectively. Thus, pemphigus could be redefined to be an anti-Dsg IgG-mediated autoimmune disease. The basic pathophysiology of pemphigus is that autoantibodies inhibit the adhesive function of Dsg and lead to the loss of cell-cell adhesion of keratinocytes with resultant blister formation.

Compelling lines of evidence, collected not only from patient serology studies, but also from experiments with mouse models, support the theory that autoimmunity to Dsg is the principal cause of pemphigus pathogenesis. Serologically, anti-Dsg1 IgG autoantibodies have been found in patients with PF, but not in normal individuals [6]. IgG prepared from PF sera induced blisters with typical PF histology when passively transferred to neonatal mice [7]. Removal of anti-Dsg1 IgG autoantibodies by immunoabsorption with baculovirus-expressed recombinant extracellular domain of Dsg1 (rDsg1) abolished the blister-forming activity of PF sera [8]. Purified anti-Dsg1 IgG on rDsg1 from PF sera induced blisters with the typical histology [8]. Similarly, anti-Dsg3 IgG autoantibodies have been found in patients with PV, but not in normal individuals [6]. IgG prepared from PV sera induced blisters with typical histology when passively transferred to neonatal mice [9]. Removal of anti-Dsg3 IgG by immunoabsorption with rDsg3 abolished the pathogenic activity of PV sera [10]. Anti-Dsg3 and anti-Dsg1 IgG autoantibodies were necessary for efficient PV blister formation in the skin of neonatal mice [11]. Furthermore, when monitored in individual patients, the titers of serum anti-Dsg1

and anti-Dsg3 IgG autoantibodies, as measured by indirect immunofluorescence or ELISA, generally correlate with disease activity [12].

Dominant Epitopes That Recognized Pemphigus IgG Autoantibodies Are on the N-Terminal Adhesive Regions of Dsg

Characterizing the Dsg binding sites of the pathogenic pemphigus autoantibodies is an essential step in understanding the pathophysiology of blister formation in pemphigus, as well as the basic molecular mechanism of Dsg-mediated cell-cell adhesion. This characterization is hindered by the fact that binding of autoantibodies to Dsg is dependent not only on amino acid sequence, but also on molecular conformation [8, 10, 13]. This dependence on molecular conformation is shown by the observation that rDsg1 and rDsg3, when expressed in baculovirus as secreted proteins, immunoabsorbs heterogeneous autoantibodies from PF and PV patients' sera, and that this immunoabsorptive activity is lost upon denaturation by Ca^{2+} chelation, acid or alkaline treatment, or boiling. Therefore, a conventional approach using variously truncated Dsg molecules is inappropriate for definition of the conformational epitopes of Dsg in pemphigus.

We used a strategy based on domain swapping and point mutations to map regions within Dsg1 and Dsg3 that constitute the conformational epitopes for PF and PV autoantibodies. Dsg1 and Dsg3 were used as swapping partners for each other because they share a similar structure but no significant cross-reactivity [5, 12]. These domain-swapped molecules were used as competitors for ELISA against the entire extracellular domain of Dsg1 or Dsg3, allowing us to measure autoantibodies against specific regions in a quantitative fashion. We performed gross mapping of the epitopes of Dsg1 and Dsg3, using four domain-swapped molecules, which divide the entire extracellular domain of Dsg1 and Dsg3 into three parts. In both PF and PV patients, major epitopes were mapped to the respective N-terminal 161 residues of Dsg1 and Dsg3 [14, 15]. These N-terminal 161 residues contained critical epitopes recognized by pathogenic IgG antibodies because the immunoabsorption of two PF sera with residues 1–161 of Dsg1 removed their activity to induce blisters in neonatal mice [15]. Although there was no single major epitope on Dsg1 and Dsg3, dominant epitopes were further mapped to residues 26–87 of Dsg1 and 25–88 of Dsg3, both of which are in the N-terminal EC1 domain [15].

Cadherins are a family of calcium-dependent cell-cell adhesion molecules that play important roles in the formation and maintenance of complex tissues [16]. Recent high-resolution crystal structure analyses of classic cadherins have provided a mechanistic basis for intermolecular cadherin interactions [17],

and more recently, the crystal structure of the entire extracellular domain of C-cadherin, which is a member of the classic cadherin family, has been deduced with a resolution of 3.1 angstroms [18]. This structure provides a new framework for understanding both the *cis* (same cell) and *trans* (juxtaposed cell) interactions of cadherin. The *trans* adhesive interface is a two-fold symmetrical interaction that is defined by a conserved tryptophan (W2) side chain at the amino-terminal, membrane-distal end of the cadherin molecule from one cell, which inserts into the hydrophobic pocket at the amino-terminal end of a cadherin molecule on an opposing cell (fig. 4). This simple two-fold symmetry provides a rationale for the generally observed homophilic specificity of cadherins, and reveals the molecular determinants of cadherin specificity. Combining the structural bases of classic cadherins and the above epitope map studies, it is indicated that the pathogenic autoantibodies in PF and PV are dominantly raised against the N-terminal adhesive interfaces of Dsg1 and Dsg3, which are the functionally important part of the molecules.

A Novel Autoimmune Mouse Model Using the Autoantigen-Deficient Mouse

To investigate the pathophysiological mechanisms and to develop therapeutic strategies, animal disease models have been playing important roles in the study of various conditions including autoimmune diseases. To examine the cellular mechanisms of autoantibody production in pemphigus, specimens from patients are naturally the best clinical material, but it is not easy to obtain sufficient amounts with proper controls. To overcome this problem, an active disease model is required. The conventional approach to develop an autoimmune mouse model is forced immunization of autoantigens in various strains of mice with various kinds of adjuvants (fig. 1a). However, this approach is empirical and immune responses are largely dependent on the strains of mice or types of adjuvants used. Furthermore, any autoimmune reaction in those mice may be transient, unlike that found in patients, and the immune system is systemically stimulated.

The major difficulty in the development of an autoimmune reaction in mice is self-tolerance, which prevents the immune system from reacting destructively against components of the living body. When lymphocytes are exposed to autocomponents during the development of the immune system, autoreacting lymphocytes are eliminated or inactivated [19, 20]. We have taken a novel approach to overcome this problem. Because self-tolerance is a technical barrier to the development of autoimmune mouse models, we thought of generating a condition where self-tolerance is not established in an antigen-specific

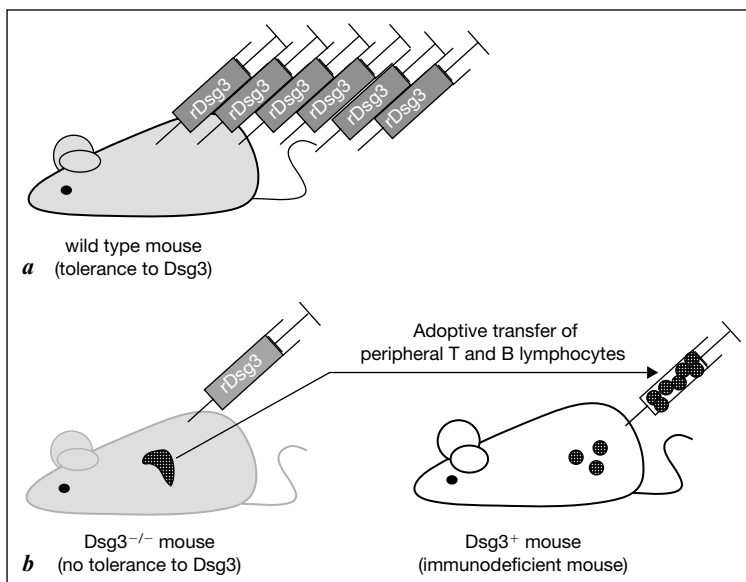


Fig. 1. Methods to develop an active disease mouse model for PV. **a** In the conventional approach, various strains of wild-type mice are repeatedly immunized with recombinant Dsg3 (rDsg3) in various adjuvants to break their immunological tolerance. None of the immunized mice developed IgG which could bind to the native Dsg3 in vivo and no IgG deposition of keratinocyte cell surfaces showed in the skin of immunized mice. **b** In a novel approach, splenocytes of Dsg3^{-/-} mice which do not acquire tolerance against Dsg3 are adoptively transferred to immunodeficient mice that express Dsg3. Recipient mice persistently produced anti-Dsg3 IgG and developed the PV phenotype.

way (fig. 1b). If it were possible to remove the antigen during the development of the immune system, or if the antigen were not present from the start, tolerance against the removed or absent molecule would not be acquired [21]. In the autoantigen knockout mouse, lymphocytes are not exposed to the defective gene product, and self-tolerance against that particular autoantigen is not established. Upon immunization with the autoantigen, the autoantigen knockout mice should elicit an immune reaction against the autoantigen. However, in the immunized knockout mice, the antigen-antibody reaction is not expected because the mice lack the target antigen. Therefore, lymphocytes from the immunized autoantigen knockout mice are adoptively transferred to wild-type mice that express the autoantigen. The transferred lymphocytes from the autoantigen knockout mice should be persistently stimulated by the endogenous autoantigen in the recipient mice and should therefore produce antibodies against the autoantigen with resultant phenotypes of the human disease.

Development of a Pemphigus Mouse Model with Persistent Pathogenic Antibody Production

To develop an active disease mouse model for PV, we took this novel approach of using autoantigen knockout mice, in the case of PV, Dsg3^{-/-} mice [21, 22]. When we immunized Dsg3^{-/-} mice with mouse recombinant Dsg3, anti-Dsg3 IgG was indeed produced. These sera were able to bind to the cell surfaces of living cultured mouse keratinocytes, indicating that the anti-Dsg3 IgG produced in Dsg3^{-/-} mice is capable of binding to the native Dsg3 on living keratinocytes. In contrast, sera from Dsg3^{+/-} littermates or wild-type mice failed to bind to the surface of living keratinocytes. These findings confirmed that Dsg3^{-/-} mice and mice expressing Dsg3 are clearly different in their ability to produce anti-Dsg3 IgG that can bind to the native Dsg3 [21].

Despite the production of anti-Dsg3 IgG, no autoimmune reaction is expected in the immunized Dsg3^{-/-} mice because they lack the target antigen. To allow the anti-Dsg3 IgG to be exposed to the antigen, we isolated splenocytes from the immunized Dsg3^{-/-} mice and transferred them into Rag-2^{-/-} immunodeficient mice that do express Dsg3 (fig. 1b). Rag-2^{-/-} mice have no mature T or B cells due to the inability to rearrange T cell receptors or immunoglobulin genes and thus are unable to produce antibodies or reject the transferred splenocytes. Circulating anti-Dsg3 IgG was detected in the sera of recipient Rag-2^{-/-} mice as early as 4 days after the transfer of Dsg3^{-/-} splenocytes, and its titer increased rapidly without further boosting by recombinant Dsg3, and reached a plateau around day 21. The circulating anti-Dsg3 IgG was detected for as long as 6 months or more. No significant reactivity against Dsg1, another desmosomal cadherin targeted in PF, was observed in these recipient mice during this period. In contrast, no circulating anti-Dsg3 IgG was detected in Rag-2^{-/-} mice given Dsg3^{+/-} splenocytes. The persistent anti-Dsg3 IgG production indicates that endogenous Dsg3 in the recipient mice stimulated the transferred Dsg3-specific lymphocytes from the immunized Dsg3^{-/-} mice in vivo.

In recipient mice with Dsg3^{-/-} splenocytes, in vivo IgG deposition was found on keratinocyte cell surfaces in stratified squamous epithelia, including the skin as well as oral and esophageal mucous membranes, just as seen in patients with PV (fig. 2c). In these mice, no IgG deposition was found in other tissues, including heart, lung, liver, kidney, stomach, and small and large intestines. These IgG binding sites correspond to the known tissue distribution of Dsg3. Histological examination of the recipient mice revealed an intraepithelial loss of cell-cell adhesion just above the basal layers, that is suprabasilar acantholysis, in the buccal mucosa, hard palate, oropharyngeal areas and the upper part of the esophagus, just as in human patients (fig. 2d). These oral erosions

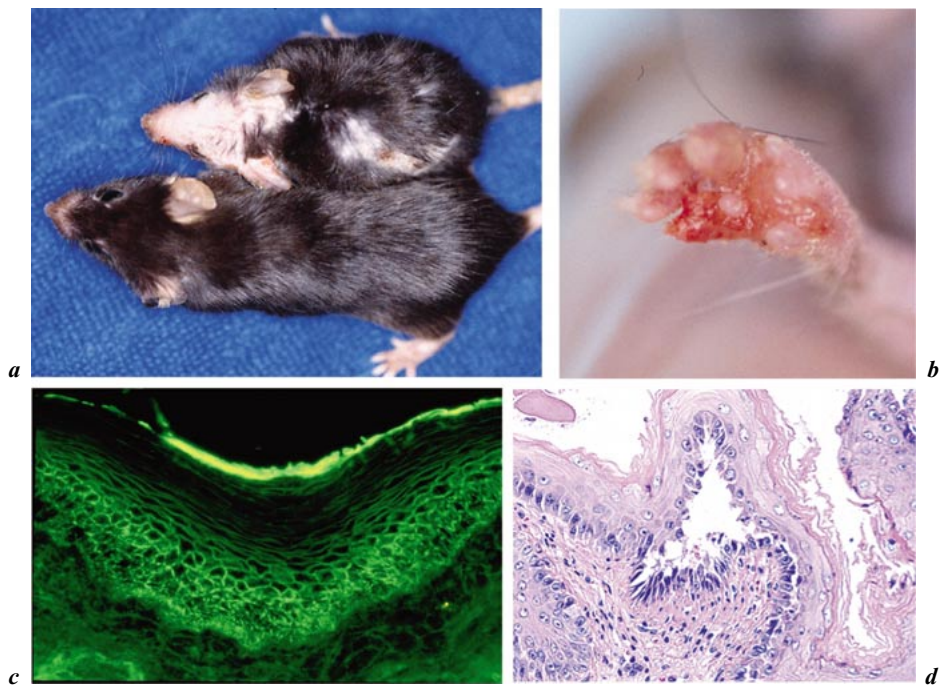


Fig. 2. Phenotype of the active disease model mice for PV. **a** The recipient mice with Dsg3^{-/-} splenocytes (upper mouse) are smaller than control mice with Dsg3^{+/-} splenocytes (lower mouse) because oral erosions inhibit food intake. **b** Some recipient mice with Dsg3^{-/-} splenocytes show crusted erosions on the paws, where constant pressure is applied. Recipient mice with Dsg3^{-/-} splenocytes show in vivo mouse IgG deposition on keratinocyte cell surfaces (**c**) and suprabasilar acantholysis (**d**) just as in patients with PV.

likely inhibited food intake, resulting in weight loss (fig. 2a). Some of the recipient mice developed crusted erosions on the skin around the snout, an area that is normally traumatized by scratching, or paws where constant pressure is applied (fig. 2b). Close histological examination revealed that the recipient mice with Dsg3^{-/-} splenocytes also exhibited eosinophilic spongiosis which is often found in patients with early lesions [23]. We also observed patchy hair loss in the recipient mice with Dsg3^{-/-} splenocytes (fig. 2a). This hair loss phenotype also persisted for over 6 months. Skin biopsy showed intense IgG deposition on the cell surface of keratinocytes surrounding the telogen hair club. Cleft formation was observed between the cells surrounding the telogen club and the basal layer of the outer root sheath epithelium. In contrast, no phenotypic or pathological changes were observed in recipient mice with

Dsg3^{+/-} splenocytes. These results indicate that the Rag-2^{-/-} recipient mice given immunized Dsg3^{-/-} splenocytes developed clinical, histologic and immunopathologic phenotypes similar to those of patients with PV [21]. This active disease model for PV provides a useful tool to investigate not only pathophysiological mechanisms of blister formation by IgG autoantibodies, but also immunological mechanisms for tolerance of B and T cells to Dsg3.

Development and Characterization of Anti-Dsg3 Monoclonal Antibodies Isolated from a Pemphigus Mouse Model

The titers of serum anti-Dsg3 IgG autoantibodies, as measured by indirect immunofluorescence or ELISA, generally correlate with disease activity when monitored in individual patients [12, 24]. However, these titers are not absolute indicators for the severity of the disease among groups of patients, and it is sometimes the case that patients with low titers of anti-Dsg3 IgG autoantibodies show severe phenotypes, while patients with high titers show mild phenotypes [12]. It is not known whether all IgG autoantibodies that bind *in vivo* to the native Dsg3 are equally pathogenic, or whether each anti-Dsg3 IgG has a distinct potency for the induction of blister formation. Furthermore, if different autoantibodies have different pathogenic activities, what defines pathogenic strength at the molecular level? The major obstacle to addressing this question is that patients' sera contain polyclonal anti-Dsg3 IgG autoantibodies that react with different parts of the Dsg3 molecule, as discussed above [14, 15]. Therefore, it is not feasible to compare the pathogenic activities of individual anti-Dsg3 IgG using patients' sera.

In order to address this issue, we used the PV model mice to generate a panel of anti-Dsg3 IgG monoclonal antibodies (AK mAbs) that could bind *in vivo* to different parts of the native Dsg3 [25]. We evaluated the pathogenic activities of these mAbs with two methods. The first method involved passive transfer of mAbs into neonatal mice, and the second method assayed ascites formation in adult mice. The passive transfer assay may be more sensitive than the ascites formation assay because highly concentrated IgG can be applied in passive transfer, while the amount of IgG that can be used in the ascites formation assay is dependent on the production rate of each hybridoma. AK19 mAb and AK23 mAb induced blister formation after passive transfer, while the remaining mAbs failed to display pathogenic activities. In the ascites formation assay, only AK23 mAb induced blisters, and the phenotypes of the mice that received AK23 hybridoma cells were virtually identical to those of PV model mice and Dsg3^{-/-} mice (fig. 3). The other AK hybridoma cells failed to induce blisters in the ascites formation assay, although the titers of circulating

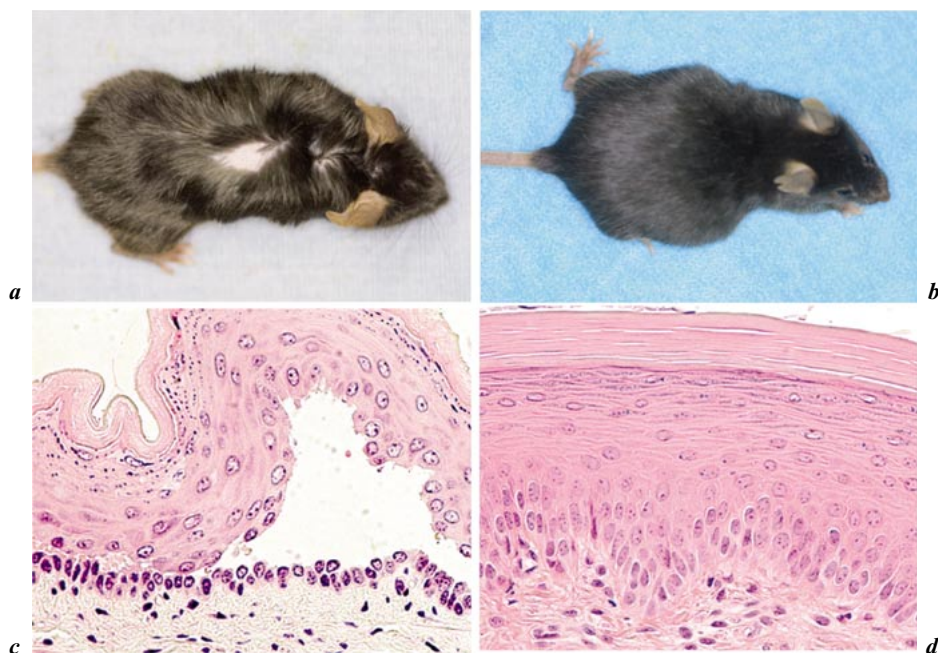


Fig. 3. Induction of PV phenotype by pathogenic AK23 mAb. When hybridoma cells that produce AK23 mAb were inoculated in Rag2^{-/-} recipient mice, the mice developed the PV phenotype including telogen hair loss (*a*) and oral erosion with suprabasilar acantholysis in histology (*c*). In contrast, when hybridoma cells that produce other AK mAbs were inoculated in Rag2^{-/-} mice, the mice developed no apparent PV phenotype (*b, d*).

AK mAbs were more than five times those of mice that were administered the AK23 hybridoma cells. These findings indicate that AK23 and AK19 are capable of inducing both the loss of cell-cell adhesion of keratinocytes and blister formation with different potencies, while the other AK mAbs apparently lack pathogenic activities. These findings demonstrate pathogenic heterogeneity among anti-Dsg3 IgG antibodies that bind *in vivo* to the native Dsg3 for the first time [25].

The epitopes of the AK mAbs were subsequently characterized by immunoprecipitation using domain-swapped as well as point-mutated Dsg1/Dsg3 molecules that were produced by baculovirus expression. Following immunoprecipitation with the domain-swapped molecules, the epitopes of AK7, AK9, AK15, AK18 and AK20, which lacked pathogenic activities, were mapped to the middle or carboxyl terminal regions (between residues 195 and 565) of the extracellular domain of mouse Dsg3. The epitopes of AK19 and

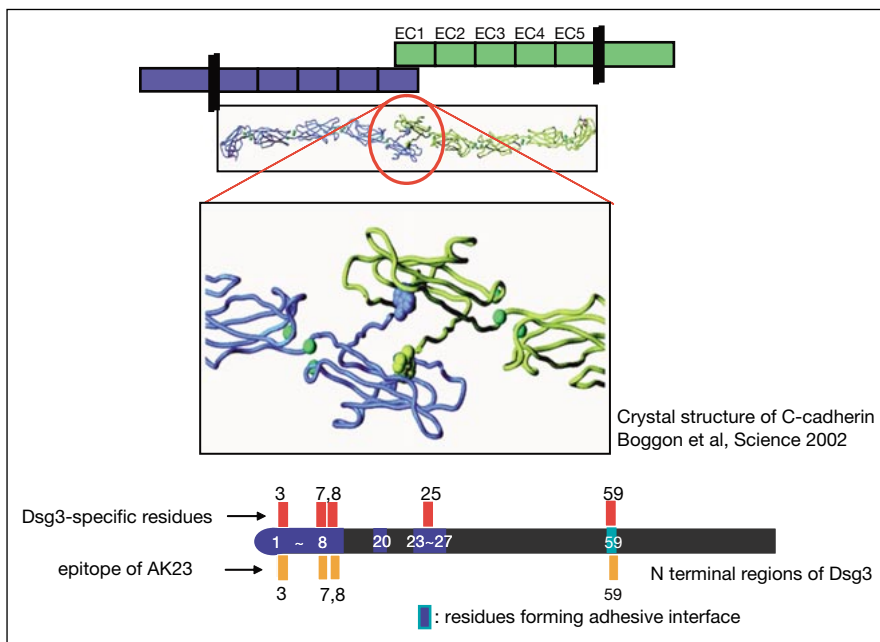


Fig. 4. Predicted three-dimensional structure of the epitope for the pathogenic AK23 mAb on Dsg3. When the amino acid sequences of Dsg3 were superimposed on the predicted structure of C-cadherin [18], the predicted residues of the adhesive interface of the W2 donor side were shown in blue-shaded boxes. Among these residues, the ones that are not conserved between Dsg3 and Dsg1 and therefore are probably involved in the determination of binding specificity of Dsg, are shown in red-shaded boxes (V3, K7, P8, T25 and D59). The epitope of AK23 mAb is shown in orange-shaded boxes (V3, K7, P8 and D59) which are located precisely on the Dsg3-specific residues of the adhesive interface.

AK23, which possessed pathogenic activities, were calcium dependent and were located in residues 89–161 and 1–63, respectively. Subsequent extensive studies with point-mutated Dsg1/Dsg3 molecules revealed that AK23 recognizes a conformational epitope that consists of the V3, K7, P8 and D59 residues of Dsg3 (fig. 4) [25].

When the amino acid sequences of Dsg3 were superimposed on the predicted structure of C-cadherin as mentioned above [18], the predicted residues for the adhesive interface of the W2 donor side were E1 to P8, P20, K23, T25, S26, D27 and D59. Among these residues, those which are not conserved between Dsg3 and Dsg1 and, therefore, probably involved in the determination of binding specificity of Dsg, are V3, K7, P8, T25 and D59. Surprisingly, residues V3, K7, P8 and D59 of the epitope of AK23, which is the most potent

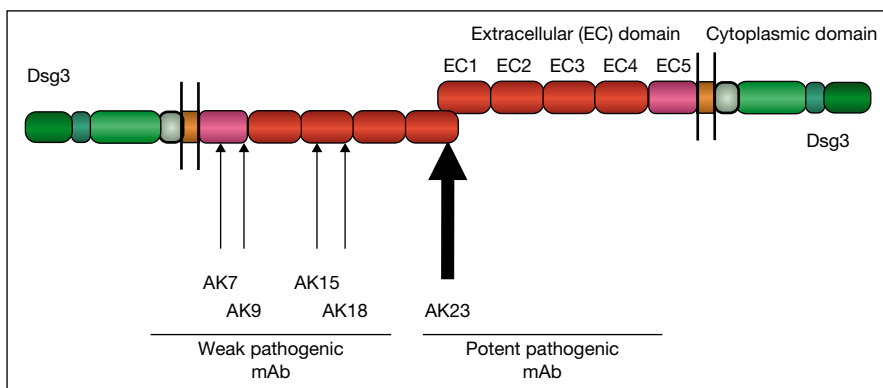


Fig. 5. Epitope map for the potent and weak pathogenic mAbs on Dsg3 molecule. The potent pathogenic AK23 mAb recognized the N-terminal adhesive interface, while weak pathogenic mAbs react with the middle to C-terminal regions of the extracellular domain of Dsg3, which includes the functionally less important parts of the molecule.

pathogenic mAb tested, were located precisely on the Dsg3-specific residues of the adhesive interface (fig. 4, 5). In contrast, the AK mAbs which lacked apparent pathogenic activities recognize the middle to C-terminal portion of the extracellular domain, a region in which no direct molecular interaction is predicted (fig. 5). Taken together, these findings indicate that the pathogenic heterogeneity among anti-Dsg3 IgG antibodies in terms of blister formation is, at least in part, explained by their epitopes, and that the amino-terminal *trans* adhesive interface may represent a critical location for blister formation by IgG antibodies in PV.

Synergistic Pathogenic Effects of Combined Mouse Anti-Dsg3 IgG mAbs on Pemphigus Blister Formation

The above findings do not necessarily exclude the possibility that antibodies against the middle to carboxyl terminal region of the extracellular domain of Dsg3 play some pathogenic role in blister formation. It is possible that the AK mAbs that lack apparent pathogenic activities per se may induce blisters when administered in combinations. A further ten anti-Dsg3 IgG mAbs were isolated from PV model mice that received naive Dsg3^{-/-} splenocytes (NAK series) [26]. The pathogenic activities of these mAbs were characterized in three different assays, passive transfer, ascites formation and in vitro dissociation [27], and their conformational epitopes were also analyzed. Although eight of ten

NAK mAbs showed pathogenic activity in the passive transfer assay, none induced the PV phenotype in the ascites formation assay, indicating that none were as potent as AK23 mAb, which could induce the PV phenotype in adult mice upon hybridoma inoculation [25]. More sensitive in vitro dissociation assays confirmed this and allowed us to rank the pathogenicity of NAK mAbs.

Because pemphigus patients' sera always contain polyclonal anti-Dsg3 IgG autoantibodies, which recognize different parts of the molecule [15], we tested the possibility that a combination of several weakly pathogenic mAbs might show a synergistic effect and induce the PV phenotype in adult mice. Indeed, when the combination of hybridoma cells producing several NAK mAbs was inoculated into Rag2^{-/-} mice, the recipients developed the PV phenotype, with weight loss, patchy hair loss and crusted erosions around the snout. The minimal combination tested that was sufficient to induce the phenotype in all mice tested was NAK1, 2, 7 and 11 mAbs or NAK2, 3, 5 and 11 mAbs. Thus, NAK mAbs recognizing the middle to C-terminal extracellular domain of Dsg3 showed synergistic effects with mAbs reacting with the N-terminal domain. These findings provide molecular evidence that anti-Dsg IgG antibodies recognizing the middle to C-terminal extracellular domains of Dsg3 can also take part in the pathogenic process of blister formation.

Efficient Production of Pathogenic Autoantibodies Requires Loss of Tolerance Against Dsg3 in Both T and B Cells

Although there is a fundamental difference between Dsg3^{-/-} mice and wild-type or Dsg3^{+/-} mice in their status of tolerance against Dsg3, it is unknown whether such self-tolerance is acquired by B cells alone, T cells alone or both in wild-type or normal mice. We thus attempted to determine whether breakdown of tolerance at the T cell level, the B cell level or both is required to produce pathogenic anti-Dsg3 IgG antibodies [28]. T and B cells were purified from Dsg3^{-/-}, Dsg3^{+/-} or wild-type mice, combined in various ways and adoptively transferred into Rag2^{-/-} recipient mice, and phenotype in each recipient mouse was examined. When the recipient mice with various combinations of T and B cells were compared, only mice with Dsg3^{-/-} T and Dsg3^{-/-} B cells showed apparent weight loss. Five of eight recipient mice with Dsg3^{-/-} T and Dsg3^{-/-} B cells showed the weight loss phenotype as well as patchy hair loss, while none of the other recipient mice with different combinations of T and B cells developed either weight loss or hair loss. Sera from all 5 recipient mice with the phenotype stained cell surface of mouse keratinocytes when they were added to culture media. In addition, these 5 mice showed IgG deposition on keratinocyte cell surface in vivo and intraepidermal

blister formation just above the basal cell layer in histology. These findings indicate that loss of tolerance against Dsg3 in T and B cells is required for efficient production of pathogenic anti-Dsg3 IgG antibodies and development of the phenotype in our mouse model for pemphigus. Since our model has similar characteristics to the human PV, it is also suggested that tolerance against Dsg3 is established in both B and T cell populations in humans and tolerance breakdown in both populations may trigger the disease onset.

Future Directions

The molecular and cellular mechanisms for tolerance against peripheral antigens or tolerance break that leads to harmful autoimmune reaction remain to be elucidated. It is not easy to solve this complex question and we need to go step by step. The PV model we developed should provide an important tool to achieve this goal. Although this model does not address the usual triggers of autoimmune diseases, it does provide a means to investigate the roles of T and B lymphocytes in perpetuating autoantibody production in the autoimmune response. In addition, this active animal model should be beneficial for evaluating various therapeutic strategies that could modulate the autoimmune response. Several studies are in progress on this line.

Acknowledgement

I acknowledge Dr. John R. Stanley for his collaboration and stimulating discussion throughout the course of the studies mentioned in this manuscript. The pemphigus mouse model was developed in collaboration with Dr. Shigeo Koyasu. The works mentioned in this article were supported by Keio Gijuku Academic Development Funds, Health Sciences Research Grants for Research on Specific Diseases from the Ministry of Health, Labour and Welfare of Japan, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- 1 Amagai M: Pemphigus; in Bologna J, Jorizzo J, Rapini R, Horn TD, Mascaró J, Mancini AJ, Salasche SJ, Saurat JH, Stingl G (eds): *Dermatology*. London, Harcourt Health Sciences, 2003, pp 449–462.
- 2 Beutner EH, Jordon RE: Demonstration of skin antibodies in sera of pemphigus vulgaris patients by indirect immunofluorescent staining. *Proc Soc Exp Biol Med* 1964;117:505–510.
- 3 Stanley JR: Cell adhesion molecules as targets of autoantibodies in pemphigus and pemphigoid, bullous diseases due to defective epidermal cell adhesion. *Adv Immunol* 1993;53:291–325.
- 4 Koch PJ, Walsh MJ, Schmelz M, Goldschmidt MD, Zimbelmann R, Franke WW: Identification of desmoglein, a constitutive desmosomal glycoprotein, as a member of the cadherin family of cell adhesion molecules. *Eur J Cell Biol* 1990;53:1–12.

- 5 Amagai M, Klaus-Kovtun V, Stanley JR: Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. *Cell* 1991;67:869–877.
- 6 Hashimoto T, Ogawa MM, Konohana A, Nishikawa T: Detection of pemphigus vulgaris and pemphigus foliaceus antigens by immunoblot analysis using different antigen sources. *J Invest Dermatol* 1990;94:327–331.
- 7 Rock B, Martins CR, Theofilopoulos AN, Balderas RS, Anhalt GJ, Labib RS, Futamura S, Rivitti EA, Diaz LA: The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). *N Engl J Med* 1989;320:1463–1469.
- 8 Amagai M, Hashimoto T, Green KJ, Shimizu N, Nishikawa T: Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. *J Invest Dermatol* 1995;104:895–901.
- 9 Anhalt GJ, Labib RS, Voorhees JJ, Beals TF, Diaz LA: Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. *N Engl J Med* 1982;306:1189–1196.
- 10 Amagai M, Hashimoto T, Shimizu N, Nishikawa T: Absorption of pathogenic autoantibodies by the extracellular domain of pemphigus vulgaris antigen (Dsg3) produced by baculovirus. *J Clin Invest* 1994;94:59–67.
- 11 Mahoney MG, Wang Z, Rothenberger KL, Koch PJ, Amagai M, Stanley JR: Explanation for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris. *J Clin Invest* 1999;103:461–468.
- 12 Ishii K, Amagai M, Hall RP, Hashimoto T, Takayanagi A, Gamou S, Shimizu N, Nishikawa T: Characterization of autoantibodies in pemphigus using antigen-specific ELISAs with baculovirus expressed recombinant desmogleins. *J Immunol* 1997;159:2010–2017.
- 13 Amagai M, Ishii K, Hashimoto T, Gamou S, Shimizu N, Nishikawa T: Conformational epitopes of pemphigus antigens (Dsg1 and Dsg3) are calcium dependent and glycosylation independent. *J Invest Dermatol* 1995;105:243–247.
- 14 Futei Y, Amagai M, Sekiguchi M, Nishifuji K, Fujii Y, Nishikawa T: Conformational epitope mapping of desmoglein 3 using domain-swapped molecules in pemphigus vulgaris. *J Invest Dermatol* 2000;115:829–834.
- 15 Sekiguchi M, Futei Y, Fujii Y, Iwasaki T, Nishikawa T, Amagai M: Dominant autoimmune epitopes recognized by pemphigus antibodies map to the N-terminal adhesive region of desmogleins. *J Immunol* 2001;167:5439–5448.
- 16 Takeichi M: Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 1991;251:1451–1455.
- 17 Shapiro L, Fannon AM, Kwong PD, Thompson A, Lehmann MS, Grubel G, Legrand JF, Als-Nielsen J, Colman DR, Hendrickson WA: Structural basis of cell-cell adhesion by cadherins. *Nature* 1995;374:327–337.
- 18 Boggon TJ, Murray J, Chappuis-Flament S, Wong E, Gumbiner BM, Shapiro L: C-cadherin ectodomain structure and implications for cell adhesion mechanisms. *Science* 2002;296:1308–1313.
- 19 MacDonald HR: Mechanisms of immunological tolerance. *Science* 1989;246:982.
- 20 Goodnow CC: Balancing immunity and tolerance: deletion and tuning lymphocyte repertoires. *Proc Natl Acad Sci USA* 1996;93:2264–2271.
- 21 Amagai M, Tsunoda K, Suzuki H, Nishifuji K, Koyasu S, Nishikawa T: Use of autoantigen knock-out mice to develop an active autoimmune disease model of pemphigus. *J Clin Invest* 2000;105:625–631.
- 22 Koch PJ, Mahoney MG, Ishikawa H, Pulkkinen L, Uitto J, Shultz L, Murphy GF, Whitaker-Menezes D, Stanley JR: Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. *J Cell Biol* 1997;137:1091–1102.
- 23 Ohyama M, Amagai M, Tsunoda K, Ota T, Koyasu S, Umezawa A, Hata J, Nishikawa T: Immunologic and histopathologic characterization of active disease mouse model for pemphigus vulgaris. *J Invest Dermatol* 2002;118:199–204.
- 24 Sams WMJ, Jordon RE: Correlation of pemphigoid and pemphigus antibody titres with activity of disease. *Br J Dermatol* 1971;84:7–13.
- 25 Tsunoda K, Ota T, Aoki M, Yamada T, Nagai T, Nakagawa T, Koyasu S, Nishikawa T, Amagai M: Induction of pemphigus phenotype by a mouse monoclonal antibody against the amino-terminal adhesive interface of desmoglein 3. *J Immunol* 2003;170:2170–2178.

- 26 Kawasaki H, Tsunoda K, Hata T, Ishii K, Yamada T, Amagai M: Synergistic pathogenic effects of combined mouse monoclonal anti-desmoglein 3 IgG antibodies on pemphigus vulgaris blister formation. *J Invest Dermatol* 2006;126:2621–2630.
- 27 Ishii K, Harada R, Matsuo I, Shirakata Y, Hashimoto K, Amagai M: In vitro keratinocyte dissociation assay for evaluation of the pathogenicity of anti-desmoglein 3 IgG autoantibodies in pemphigus vulgaris. *J Invest Dermatol* 2005;124:939–946.
- 28 Tsunoda K, Ota T, Suzuki H, Ohyama M, Nagai T, Nishikawa T, Amagai M, Koyasu S: Pathogenic autoantibody production requires loss of tolerance against desmoglein 3 in both T and B cells in experimental pemphigus vulgaris. *Eur J Immunol* 2002;32:627–633.

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Pemphigus Foliaceus

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Abstract

Pemphigus foliaceus (PF) and its endemic form fogo selvagem (FS) are autoimmune diseases characterized clinically by transient cutaneous superficial blisters. As opposed to pemphigus vulgaris (PV), patients lack mucosal involvement. Acantholysis in the upper epidermis is appreciated histologically. The serologic hallmark of PF and FS is the demonstration of IgG autoantibodies against the cell surface of keratinocytes. The specific target of these autoantibodies is desmoglein (Dsg) 1, one of the four known desmosomal cadherins, a family of transmembrane glycoproteins that play an important role in the dynamic regulation of intercellular adhesion. Compelling evidence has been compiled suggesting anti-Dsg1 autoantibodies in patients with PF and FS are pathogenic. The mechanism by which anti-Dsg autoantibodies induce loss of cell-cell adhesion in PF is under active investigation and is beginning to be elucidated. The study of the pathogenesis of PF and FS provides a unique opportunity to uncover insights that may contribute to our greater understanding of autoimmunity.

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Pemphigus foliaceus (PF) and its endemic form fogo selvagem (FS) are autoimmune diseases characterized clinically by transient cutaneous superficial blisters. These blisters tend to be fragile to the point that often only scaly, crusted erosions are appreciated on physical examination. Lesions are typically in a seborrheic distribution and may be mistaken for impetigo. The Nikolsky sign, a well-described clinical sign in which blistering or denudation of the epidermis is elicited by lateral pressure, is present. As opposed to pemphigus vulgaris (PV), patients lack mucosal involvement. The disease often remains localized for many years, but may progress in some patients to an erythrodermic exfoliative dermatitis. Patients may describe a burning sensation in involved areas. Acantholysis in the upper epidermis is appreciated histologically. By direct immunofluorescence, IgG autoantibodies are seen within intercellular

spaces between keratinocytes in lesional and perilesional tissue [1]. Circulating patient IgG targeting the intercellular spaces may also be demonstrated using indirect immunofluorescence. Titers of circulating autoantibodies are well correlated with disease activity.

The serologic hallmark of PF and FS is the demonstration of IgG autoantibodies against the cell surface of keratinocytes. The specific target of these autoantibodies is desmoglein (Dsg) 1, one of the four known desmosomal cadherins, a family of transmembrane glycoproteins that play a particularly important role in the dynamic regulation of intercellular adhesion. Desmosomal cadherins and classical cadherins share high sequence homology and structural similarities in their ectodomain, a region composed of four cadherin repeats (EC1–EC4) and a membrane-proximal region (EC5). In adherens junctions, the ectodomains of classical cadherins expressed between adjacent cells interact homophilically. The cytoplasmic tails of these cadherins are associated with the actin cytoskeleton network via a complex of catenins. In desmosomes, adhesion is mediated by homophilic or heterophilic interactions of the ectodomains of two types of desmosomal cadherins, Dsg and desmocollins. The cytoplasmic domain of the desmosomal cadherins connects to the intermediate filament network through desmosomal plaque proteins including plakoglobin, plakophilins and desmoplakins.

Compelling evidence has been compiled suggesting anti-Dsg1 autoantibodies in patients with PF and FS are pathogenic. Passive transfer of patients' IgG to neonatal mice results in murine clinical and histologic findings that mirror the human disease [2, 3]. Furthermore, immunoadsorption of PF sera with Dsg1 extracellular domain eliminates the pathogenic response of these sera [4]. However, transient neonatal disease, commonly observed in the setting of maternal PV, has very rarely been described in PF despite the demonstration of passively transferred maternal antibodies to Dsg1 bound to neonatal epidermis [5, 6]. This seeming paradox has been explained by demonstration of the coexpression of Dsg3 in the superficial layers of neonatal epidermis [7]. In this sense, the distribution of Dsg in neonatal skin resembles that of adult mucosa.

The Dsg compensation hypothesis, the idea that one Dsg can compensate for loss of function of another, has been proposed to explain the observed clinical differences in the character and distribution of blisters in patients with PV versus PF, and has been validated in a number of studies [8, 9]. The expression of desmosomal cadherins varies within the human epidermis. In adult skin, Dsg1 is present throughout the epidermis, whereas Dsg3 is present only in the basal and immediate suprabasal layers. In patients with PF and FS, pathogenic anti-Dsg1 autoantibodies result in blister formation only in the superficial epidermis, which contains Dsg1 without coexpressed Dsg3. In the unaffected deep epidermis, the presence of Dsg3 is believed to compensate for the loss of function of Dsg1.

Recently, circulating nonpathogenic autoantibodies targeting Dsg4, a newly identified member of the Dsg family expressed in the suprabasal layers of the epidermis, have been demonstrated in PF and mucocutaneous PV patient sera [10]. Additionally, antibodies targeting E-cadherin, a classical cadherin expressed in all layers of the epidermis, have also recently been demonstrated in the sera of PF, FS and mucocutaneous PV patients [11]. Both anti-Dsg4 and anti-E-cadherin autoantibodies have been shown to cross-react with the extracellular domain of Dsg1. The pathogenicity of anti-E-cadherin autoantibodies is not yet known.

While PF and FS are identical clinically, histologically and serologically, the epidemiological features of FS are distinct. The vast majority of PF cases in North America, Europe and Asia are sporadic, without evidence of geographic clustering. The disease has a mean age of onset of 50–60 years. FS, however, is a disease of individuals dedicated to outdoor activities, and cases typically exhibit geographic and familial clustering. FS is endemic amongst poor laborers of all races and both sexes living in certain endemic regions of Brazil. FS tends to affect individuals in the second and third decades of life. Other forms of endemic PF have been reported in Colombia [12], Peru [13] and Tunisia [14].

The Amerindian reservation of Limao Verde, located in the state of Mato Grosso do Sul, Brazil, is the home of approximately 1,200 members of the Terena tribe of Amerindians and an active focus of FS, exhibiting a 3.4% prevalence of the disease [15]. For the last 12 years, clinical, epidemiological and serological data from FS patients, as well as clinically normal individuals residing in and around this settlement, have been systematically collected. It has been demonstrated that the autoantibody response against Dsg1 in healthy individuals from Limao Verde and neighboring communities is common and directly related to proximity to this reservation [16].

Seroepidemiologic observations of individuals living in endemic areas of FS have demonstrated the conversion of IgG1 autoantibodies targeting the EC5 domain of Dsg1 during the preclinical stage to a predominantly IgG4-mediated autoantibody response against the Dsg1 EC1 and EC2 domains with the onset of clinically active disease. Using a sensitive and specific subclass ELISA with recombinant Dsg1, Warren et al. [17] demonstrated that while many clinically normal subjects living in endemic areas of FS possess an anti-Dsg1 IgG1 and IgG4 response, FS patients exhibit a mean 19.3-fold higher IgG4 response. Weak anti-Dsg1 IgG4 responses were observed in FS patients in clinical remission, whereas a 74.3-fold higher IgG4 response was observed in patients with active disease. Finally, in five FS patients in whom blood samples were available prior to the onset of clinical disease, a mean 103.08-fold rise in IgG4 was associated with onset of clinical disease, but only a mean 3.45-fold rise in IgG1,

suggesting that acquisition of an IgG4 response is a key step in the development of clinical disease.

The work by Li et al. [18] also suggests that the conversion from preclinical stage to disease state may be dependent upon the epitope specificity of anti-Dsg1 autoantibodies. By immunoprecipitation coupled to immunoblotting using constructed recombinant antigens, sera from FS patients in the preclinical stage were shown to recognize epitopes on the COOH-terminal EC5 domain of Dsg1. Disease onset was associated with the emergence of antibodies specific for epitopes on the NH₂-terminal EC1 and EC2 domains. Furthermore, sera from FS patients with active disease recognized the EC1 and/or EC2 domains, whereas the reactivity of sera from FS patients in remission was restricted to EC5. This observation suggests that intramolecular epitope spreading may modulate onset, remission and relapse of blistering in FS.

FS is strongly associated with certain HLA-DRB1 alleles, such as DRB1*0404, *1402 and *1406 [19]. Individuals carrying these alleles have a relative risk as high as 14. Work by Lin et al. [20] demonstrated that T cells from the majority of FS patients proliferate when stimulated with recombinant Dsg1. Additionally, Dsg1-reactive T cell lines and clones derived from FS patients were shown to express a CD4-positive memory T cell phenotype and a T helper 2-like cytokine profile (a profile known to induce B cells to secrete IgG4). Finally, specific Dsg1-induced T cell proliferation was shown to be restricted to HLA-DR molecules.

Interestingly, autoantibodies against Dsg3 have also been detected in sera from patients with FS [21]. Moreover, a high prevalence of IgG autoantibodies against Dsg3 was recently demonstrated amongst healthy individuals living in an endemic area of FS [22]. Both patients and clinically normal subjects lacked mucosal disease, but may represent a population potentially at risk for developing an endemic form of PV.

Epidemiological studies have shown that the incidence of FS tends to decrease as living conditions of endemic populations improve, as observed in the Brazilian states of Sao Paulo and Parana over the last 4 decades [23, 24]. Additional seroepidemiological studies suggest that exposure to blood-feeding arthropods such as simuliids, bed bugs and reduvid bugs, as well as poor housing are potential risk factors of FS [25]. Furthermore, the sera of patients with leishmaniasis, onchocerciasis and Chagas disease contain significant titers of anti-Dsg1 autoantibodies, suggesting that arthropod antigens may cross-react with epidermal Dsg1, thus triggering autoantibody formation in exposed individuals [26]. These observations have led investigators to suspect that the autoimmune response in FS is triggered by an as yet unknown environmental factor(s). It should be stressed, however, that attempts to isolate or directly associate FS to bacterial and viral infections have been unsuccessful to date.

Molecular Mechanism of Acantholysis

It is quite clear from the studies cited above that pathogenic antibodies that target the desmosome protein Dsg1 are responsible for blister formation in PF and FS. The mechanism by which anti-Dsg autoantibodies induce loss of cell-cell adhesion in both PV and PF is under active investigation and is beginning to be elucidated. Several concepts have been proposed to explain the ability of anti-Dsg antibodies to induce loss of cell-cell adhesion and include steric hindrance [27], proteolytic activity [28–30], keratinocyte signal transduction [31–38], Dsg endocytosis [39, 40], altered desmosome protein synthesis and/or degradation [41], and apoptosis [42–45].

The identification by Stanley and colleagues [46–48] of Dsg1 and Dsg3 as the autoantibody targets in PF and PV, respectively, made it possible to probe the mechanisms by which the autoimmune response caused loss of cell-cell adhesion. Recognizing the structural similarity between Dsgs and the classical cadherins, and that Dsgs were components of the desmosome cell-cell adhesion complex, led to early speculation that anti-Dsg antibodies disrupted cell-cell adhesion by blocking the sites on Dsg required to maintain adhesive interactions between two adjacent (either *cis* or *trans*) proteins. This steric mechanism of acantholysis has several attractive features, including its simplicity. In support of this mechanism, Amagai and coworkers [27] used immunoelectron microscopy to map the binding of PV autoantibody-Dsg interactions on keratinocytes in vivo using their active mouse model. The distribution of PV IgG binding in nonacantholytic areas of murine skin was found to be distributed throughout the extracellular portion of the desmosome, indicating that PV IgG can readily access the target antigen throughout intact desmosomes. However, steric hindrance may not be sufficient to explain how pemphigus IgG cause acantholysis. For example, using immunofluorescent techniques, Kowalczyk and coworkers [40] observed time-dependent endocytosis of cell surface-bound PV IgG Dsg3. The colocalization of these internalized complexes with markers for endosomes and lysosomes suggested that PV IgG resulted in the internalization and degradation of Dsg3. Furthermore, the PV IgG disruption of keratinocyte adhesion and Dsg3 internalization was temperature dependent, occurring at 37°C, but not when cells were incubated at 4°C. Importantly, PV IgG still bound to the cell surface at 4°C. The temperature dependence of internalization and disruption of adhesion, and the temperature independence of PV IgG binding to the cell surface Dsg3 demonstrate that events beyond simple binding of PV IgG to Dsg3 are required for acantholysis. Thus, steric hindrance of adhesive interactions is not sufficient to fully explain the loss of adhesion. Furthermore, in a recent report, preincubation of Dsg1-coated beads with pathogenic PF IgG failed to block the binding of beads to HaCat cells, whereas

addition of PF IgG to HaCat cells bound to Dsg1-coated beads resulted in bead release [49]. This observation similarly suggests that cellular events beyond simple steric hindrance of binding are required for pemphigus IgG to disrupt adhesive interactions at the desmosomal cadherin interface.

Activation of proteolytic enzymes by autoantibodies has also been proposed as a mechanism for pemphigus autoantibody-induced acantholysis. One function of IgG is to fix complement and activate a variety of inflammatory cell-mediated proteinases. For example, in the human autoimmune blistering disease bullous pemphigoid (BP), IgG directed against the hemidesmosome-associated protein BP180 causes subepidermal blistering by activation of proteolytic enzymes that ultimately results in proteolysis of BP180 with a direct disruption of its ability to mediate cell-substrate adhesion. Combining the passive transfer mouse model of BP [50, 51] with mouse knockout and transgenic technology, Liu and colleagues [50, 52, 53] have defined a series of steps subsequent to IgG binding to BP180 that are required for autoantibody-induced subepidermal blistering. Subsequent to the binding of pathogenic IgG to the NC16A extracellular epitope of BP180, complement is fixed and activated, releasing C5a [54]. Through a series of intermediate steps involving mast cell infiltration and degranulation [55], neutrophils infiltrate the skin and also degranulate [56–58], releasing the proteinases gelatinase B and neutrophil elastase [59, 60]. In BP, direct cleavage of the target antigen by neutrophil elastase is the terminal step in subepidermal blistering.

In a very elegant series of experiments, Stanley and coworkers [61–63] have demonstrated that proteolytic cleavage of Dsg1 was responsible for blistering seen in bullous impetigo and staphylococcal scalded skin syndrome. Noting the histopathologic similarities between PF and bullous impetigo, they subsequently demonstrated that the staphylococcal exotoxin was in fact a serine proteinase that specifically cleaved the extracellular region of Dsg1. Plasminogen and elastase activation and cleavage of Dsg have also been proposed as mechanisms by which pemphigus autoantibodies induce loss of adhesion. In contrast to BP, proteolytic activity does not appear to be essential for acantholysis in pemphigus; plasminogen or elastase knockout mice still develop blisters after passive transfer of PV or PF autoantibodies [64]. Furthermore, immunoglobulin effector functions are not required for antibody-induced acantholysis, since Fab and Fab' fragments derived from PV IgG lack immunoglobulin effector functions such as the ability to fix complement, yet still induce acantholysis when passively transferred to neonatal mice [30, 65]. Monovalent PV and PF IgG Fab' and anti-Dsg single-chain variable region fragments, which are incapable of cross-linking desmosome cadherins, are pathogenic [65–67].

The data suggest that binding of pemphigus antibody to Dsg is the initial event that triggers acantholysis; however, additional temperature-dependent

events within the target keratinocyte are required for cells to lose adhesion. Kitajima's group [31] was the first to investigate pemphigus IgG-mediated signal transduction in target keratinocytes. They reported a variety of intracellular signaling events initiated in keratinocyte tissue culture after exposure to either pemphigus sera or IgG fractions, including phospholipase C-dependent increases in intracellular calcium flux and inositol tris-phosphate [32] and changes in the subcellular distribution of protein kinase C [33]. These observations suggested that in addition to functioning as cell adhesion complexes, desmosomes might also function as signal transducers.

Our group then began to use pemphigus autoantibodies as a tool to investigate the relationship between changes in desmosome adhesion and signal transduction [38]. Our observations not only demonstrated that PV and PF IgG activate desmosome signaling, but that inhibition of signaling prevents acantholysis, indicating that signaling likely is causal to the mechanism of acantholysis. Pathogenic PV Dsg3 autoantibodies were used to initiate desmosome signaling in human keratinocyte cell cultures in the presence of [^{32}P] H_3PO_4 . Two-dimensional gel electrophoresis of ^{32}P -labeled keratinocyte extracts was used to identify rapid dose- and time-dependent changes in protein phosphorylation. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy of in-gel tryptic digests was used for phosphoprotein identification. Heat shock protein 27 (HSP27) and p38 mitogen-activated protein kinase (p38) were identified as proteins rapidly phosphorylated in response to PV IgG. HSP27 regulates both actin [68–70] and intermediate filaments [71, 72], whereas p38-mediated phosphorylation of HSP27 has been shown to regulate the cytoskeleton [73–75]. Missense mutations in HSP27 lead to disrupted neurofilament assembly and cause the neuromuscular disorder Charcot-Marie-Tooth disease as well as distal hereditary motor neuropathy providing additional support for the role of HSP27 in intermediate filament regulation [72]. Additionally, HSP27 may have a role in apoptosis; disruption of HSP27 binding to cytochrome c has been shown to activate caspase and induce apoptosis [76], and several recent reports describe increased keratinocyte apoptosis in PV [42–45].

We then demonstrated that inhibition of p38 activity prevented PV IgG-induced HSP27 phosphorylation, keratin filament retraction and actin reorganization [38]. These observations show that PV IgG binding to Dsg3 activates desmosomal signal transduction cascades leading to p38 and HSP27 phosphorylation, as well as cytoskeletal reorganization, supporting a mechanistic role for signaling in PV IgG-induced acantholysis. We proposed that targeting desmosome signaling via inhibition of p38 and HSP27 phosphorylation could be used to treat PV.

Using the passive transfer mouse model of PV, we then showed that PV IgG significantly induces both p38 and HSP25 phosphorylation *in vivo*, and

that either of the two p38 inhibitors SB203580 and SB202190 block phosphorylation and prevent PV blistering in vivo [77]. Similarly, we showed that PF IgG also activates p38 and HSP25 phosphorylation in vivo and that p38 inhibitors block PF IgG-induced acantholysis in the passive transfer mouse model [78]. Our observations in the PV and PF IgG passive transfer models demonstrate that p38 inhibitors can prevent skin blistering by inhibiting PV and PF IgG-activated signaling in epidermal cells targeted by pemphigus autoantibodies. Indeed, the role of p38 and intracellular signaling in pemphigus acantholysis has recently been confirmed by other investigators [49, 79].

Collectively, these observations suggest a mechanism by which pemphigus IgG initiates acantholysis. The initial event, binding of pathogenic IgG to the target Dsg, likely initiates a structural transition in the target protein. The precise nature of this structural change is unknown, but may include steric hindrance of heterophilic or homophilic binding between desmosomal cadherins within the same or apposing cells, disruption of desmosomal protein-protein interactions, and/or conformational changes within the target Dsg molecule. The structural transition within the desmosome may then result in increased p38 phosphorylation by several mechanisms, including activation of protein kinase(s), inhibition of protein phosphatase(s), and/or exposure of substrate to kinase. Signaling through single-pass transmembrane receptors is often propagated by either dissociating or bringing together juxtaposing domains of adjacent polypeptides on the cytoplasmic surface of the membrane to generate binding sites for signaling cassettes or for protein posttranslational modification. Analogous to other signal pass transmembrane receptors, the propagation of the signal induced by pemphigus IgG binding to the Dsg ectodomain may be via modulation of a docking plexus on the cytoplasmic surface of the membrane. Likely structural components for this docking plexus include the Dsg cytoplasmic tail as well as associated proteins. Plakoglobin may be a component of this docking plexus. It is known that catenins function as adaptor proteins mediating interactions of structural, signaling and transcriptional regulatory proteins. Plakoglobin is a component of adherens junctions, linking E-cadherin to the actin-binding protein α -catenin, and of desmosomes, linking Dsg to desmoplakin, plakophilin, and in turn, keratin intermediate filaments. The suggestion that plakoglobin's association with the adherens junction nucleates' formation of desmosomes may explain why plakoglobin is a component of both adherens junctions and desmosomes [80, 81], whereas β -catenin is limited to adherens junctions. Indeed, plakoglobin's cell adhesion and signaling functions are regulated by posttranslational modification including phosphorylation [82, 83] and O-glycosylation [84]. Plakoglobin may have a critical role in PV IgG-mediated acantholysis. Plakoglobin knockout keratinocytes do not retract their keratin intermediate filaments or lose adhesion when exposed to PV IgG

[35]. Disruption of molecular interactions and the cytoplasmic desmosome interface, collapse of the cytoskeleton, Dsg endocytosis, apoptosis, and additional intracellular events may occur sequentially and/or act collectively or synergistically to result in loss of cellular adhesion. The challenge for the future will be to develop a precise description of this coordinated and complex biological response.

References

- 1 Beutner EH, Prigenzi LS, Hale W, Leme Cde A, Bier OG: Immunofluorescent studies of autoantibodies to intercellular areas of epithelia in Brazilian pemphigus foliaceus. *Proc Soc Exp Biol Med* 1968;127:81–86.
- 2 Anhalt GJ, Labib RS, Voorhees JJ, Beals TF, Diaz LA: Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. *N Engl J Med* 1982;306:1189–1196.
- 3 Roscoe JT, Diaz L, Sampaio SA, Castro RM, Labib RS, Takahashi Y, Patel H, Anhalt GJ: Brazilian pemphigus foliaceus autoantibodies are pathogenic to BALB/c mice by passive transfer. *J Invest Dermatol* 1985;85:538–541.
- 4 Amagai M, Hashimoto T, Green KJ, Shimizu N, Nishikawa T: Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. *J Invest Dermatol* 1995;104:895–901.
- 5 Walker DC, Kolar KA, Hebert AA, Jordon RE: Neonatal pemphigus foliaceus. *Arch Dermatol* 1995;131:1308–1311.
- 6 Hirsch R, Anderson J, Weinberg JM, Burnstein P, Echt A, Fermin J, Heilman ER, Laude TA: Neonatal pemphigus foliaceus. *J Am Acad Dermatol* 2003;49:S187–S189.
- 7 Wu H, Wang ZH, Fakhrazadeh S, Amagai M, Ishikawa H, Uitto J, Stanley JR: Expression of desmoglein 3 in the superficial epidermis of neonatal mice protects against blisters induced by pemphigus foliaceus antibodies. *J Invest Dermatol* 2000;114:778.
- 8 Mahoney MG, Wang Z, Rothenberger K, Koch PJ, Amagai M, Stanley JR: Explanations for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris. *J Clin Invest* 1999;103:461–468.
- 9 Hanakawa Y, Matsuyoshi N, Stanley JR: Expression of desmoglein 1 compensates for genetic loss of desmoglein 3 in keratinocyte adhesion. *J Invest Dermatol* 2002;119:27–31.
- 10 Nagasaka T, Nishifuji K, Ota T, Whittock NV, Amagai M: Defining the pathogenic involvement of desmoglein 4 in pemphigus and staphylococcal scalded skin syndrome. *J Clin Invest* 2004;114:1484–1492.
- 11 Evangelista F, Dasher DA, Diaz LA, Li N: The prevalence of autoantibodies against E-cadherin in pemphigus. *J Invest Dermatol* 2006;126:S9.
- 12 Robledo MA, Prada S, Jaramillo D, Leon W: South American pemphigus foliaceus: study of an epidemic in El Bagre and Nechi, Colombia 1982 to 1986. *Br J Dermatol* 1988;118:737–744.
- 13 Heimgartner E, De Heimgartner V: Experience with endemic dermatological diseases in the Peruvian wilderness: mucocutaneous leishmaniasis and Brazilian foliaceous pemphigus. *Med Cutan Ibero Lat Am* 1976;4:1–6.
- 14 Morini JP, Jomaa B, Gorgi Y, Saguem MH, Nouria R, Roujeau JC, Revuz J: Pemphigus foliaceus in young women: an endemic focus in the Sousse area of Tunisia. *Arch Dermatol* 1993;129:69–73.
- 15 Hans-Filho G, dos Santos V, Katayama JH, Aoki V, Rivitti EA, Sampaio SA, Friedman H, Moraes JR, Moraes ME, Eaton DP, et al: An active focus of high prevalence of fogo selvagem on an Amerindian reservation in Brazil. Cooperative Group on Fogo Selvagem Research. *J Invest Dermatol* 1996;107:68–75.
- 16 Warren SJ, Lin MS, Giudice GJ, Hoffmann RG, Hans-Filho G, Aoki V, Rivitti EA, Santos V, Diaz LA: The prevalence of antibodies against desmoglein 1 in endemic pemphigus foliaceus in Brazil. Cooperative Group on Fogo Selvagem Research. *N Engl J Med* 2000;343:23–30.

- 17 Warren SJ, Arteaga LA, Rivitti EA, Aoki V, Hans-Filho G, Qaqish BF, Lin MS, Giudice GJ, Diaz LA: The role of subclass switching in the pathogenesis of endemic pemphigus foliaceus. *J Invest Dermatol* 2003;120:104–108.
- 18 Li N, Aoki V, Hans-Filho G, Rivitti EA, Diaz LA: The role of intramolecular epitope spreading in the pathogenesis of endemic pemphigus foliaceus (fogo selvagem). *J Exp Med* 2003;197:1501–1510.
- 19 Moraes ME, Fernandez-Vina M, Lazaro A, Diaz LA, Filho GH, Friedman H, Rivitti E, Aoki V, Stastny P, Moraes JR: 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;49:35–40.
- 20 Lin MS, Fu CL, Aoki V, Hans-Filho G, Rivitti EA, Moraes JR, Moraes ME, Lazaro AM, Giudice GJ, Stastny P, et al: Desmoglein-1-specific T lymphocytes from patients with endemic pemphigus foliaceus (fogo selvagem). *J Clin Invest* 2000;105:207–213.
- 21 Arteaga LA, Prisyanyh PS, Warren SJ, Liu Z, Diaz LA, Lin MS: A subset of pemphigus foliaceus patients exhibits pathogenic autoantibodies against both desmoglein-1 and desmoglein-3. *J Invest Dermatol* 2002;118:806–811.
- 22 Hilario-Vargas J, Dasher DA, Li N, Aoki V, Hans-Filho G, dos Santos V, Qaqish BF, Rivitti EA, Diaz LA: Prevalence of anti-desmoglein-3 antibodies in endemic regions of fogo selvagem in Brazil. *J Invest Dermatol* 2006;126:2044–2048.
- 23 Empinotti JC, Diaz LA, Martins CR, Rivitti EA, Sampaio SA, Lombardi C, Sanches JA: Endemic pemphigus foliaceus in western Parana, Brazil (1976–1988). Cooperative Group for Fogo Selvagem Research. *Br J Dermatol* 1990;123:431–437.
- 24 Lombardi C, Borges PC, Chaul A, Sampaio SA, Rivitti EA, Friedman H, Martins CR, Sanches JA Jr, Cunha PR, Hoffmann RG, et al: Environmental risk factors in endemic pemphigus foliaceus (Fogo selvagem). Cooperative Group on Fogo Selvagem Research. *J Invest Dermatol* 1992;98:847–850.
- 25 Aoki V, Millikan RC, Rivitti EA, Hans-Filho G, Eaton DP, Warren SJ, Li N, Hilario-Vargas J, Hoffmann RG, Diaz LA: Environmental risk factors in endemic pemphigus foliaceus (fogo selvagem). *J Invest Dermatol Symp Proc* 2004;9:34–40.
- 26 Diaz LA, Arteaga LA, Hilario-Vargas J, Valenzuela JG, Li N, Warren S, Aoki V, Hans-Filho G, Eaton D, dos Santos V, et al: Anti-desmoglein-1 antibodies in onchocerciasis, leishmaniasis and Chagas disease suggest a possible etiological link to fogo selvagem. *J Invest Dermatol* 2004;123:1045–1051.
- 27 Shimizu A, Ishiko A, Ota T, Tsunoda K, Amagai M, Nishikawa T: IgG binds to desmoglein 3 in desmosomes and causes a desmosomal split without keratin retraction in a pemphigus mouse model. *J Invest Dermatol* 2004;122:1145–1153.
- 28 Woo TY, Hogan VA, Patel H, Anhalt GJ, Labib RS, Voorhees JJ, Diaz LA: Specificity and inhibition of the epidermal cell detachment induced by pemphigus IgG in vitro. *J Invest Dermatol* 1983;81:115s–121s.
- 29 Hashimoto K, Shafran KM, Webber PS, Lazarus GS, Singer KH: Anti-cell surface pemphigus autoantibody stimulates plasminogen activator activity of human epidermal cells. *J Exp Med* 1983;157:259–272.
- 30 Anhalt GJ, Till GO, Diaz LA, Labib RS, Patel HP, Eaglstein NF: Defining the role of complement in experimental pemphigus vulgaris in mice. *J Immunol* 1986;137:2835–2840.
- 31 Seishima M, Esaki C, Osada K, Mori S, Hashimoto T, Kitajima Y: Pemphigus IgG, but not bullous pemphigoid IgG, causes a transient increase in intracellular calcium and inositol 1,4,5-triphosphate in DJM-1 cells, a squamous cell carcinoma line. *J Invest Dermatol* 1995;104:33–37.
- 32 Esaki C, Seishima M, Yamada T, Osada K, Kitajima Y: Pharmacologic evidence for involvement of phospholipase C in pemphigus IgG-induced inositol 1,4,5-trisphosphate generation, intracellular calcium increase, and plasminogen activator secretion in DJM-1 cells, a squamous cell carcinoma line. *J Invest Dermatol* 1995;105:329–333.
- 33 Osada K, Seishima M, Kitajima Y: Pemphigus IgG activates and translocates protein kinase C from the cytosol to the particulate/cytoskeleton fractions in human keratinocytes. *J Invest Dermatol* 1997;108:482–487.

- 34 Aoyama Y, Owada MK, Kitajima Y: A pathogenic autoantibody, pemphigus vulgaris-IgG, induces phosphorylation of desmoglein 3, and its dissociation from plakoglobin in cultured keratinocytes. *Eur J Immunol* 1999;29:2233–2240.
- 35 Caldelari R, de Bruin A, Baumann D, Suter MM, Bierkamp C, Balmer V, Muller E: A central role for the armadillo protein plakoglobin in the autoimmune disease pemphigus vulgaris. *J Cell Biol* 2001;153:823–834.
- 36 Nguyen VT, Ndoe A, Shultz LD, Pittelkow MR, Grando SA: Antibodies against keratinocyte antigens other than desmogleins 1 and 3 can induce pemphigus vulgaris-like lesions. *J Clin Invest* 2000;106:1467–1479.
- 37 Nguyen VT, Arredondo J, Chernyavsky AI, Kitajima Y, Pittelkow M, Grando SA: Pemphigus vulgaris IgG and methylprednisolone exhibit reciprocal effects on keratinocytes. *J Biol Chem* 2004;279:2135–2146.
- 38 Berkowitz P, Hu P, Liu Z, Diaz LA, Enghild JJ, Chua MP, Rubenstein DS: Desmosome Signaling: Inhibition of p38MAPK prevents pemphigus vulgaris IgG-induced cytoskeleton reorganization. *J Biol Chem* 2005;280:23778–23784.
- 39 Aoyama Y, Kitajima Y: Pemphigus vulgaris-IgG causes a rapid depletion of desmoglein 3 (Dsg3) from the Triton X-100 soluble pools, leading to the formation of Dsg3-depleted desmosomes in a human squamous carcinoma cell line, DJM-1 cells. *J Invest Dermatol* 1999;112:67–71.
- 40 Calkins CC, Setzer SV, Jennings JM, Summers S, Tsunoda K, Amagai M, Kowalczyk AP: Desmoglein endocytosis and desmosome disassembly are coordinated responses to pemphigus autoantibodies. *J Biol Chem* 2006;281:7623–7634.
- 41 Berkowitz P, Hu P, Prisyanyh PS, Diaz LA, Rubenstein DS: Desmoglein-3 trafficking through keratinocyte desmosomes suggests dynamic instability: a kinetic model for PV IgG acantholysis. *J Invest Dermatol* 2005;124:A28.
- 42 Wang X, Bregegere F, Frusic-Zlotkin M, Feinmesser M, Michel B, Milner Y: Possible apoptotic mechanism in epidermal cell acantholysis induced by pemphigus vulgaris autoimmunoglobulins. *Apoptosis* 2004;9:131–143.
- 43 Wang X, Bregegere F, Soroka Y, Frusic-Zlotkin M, Milner Y: Replicative senescence enhances apoptosis induced by pemphigus autoimmune antibodies in human keratinocytes. *FEBS Lett* 2004;567:281–286.
- 44 Pelacho B, Natal C, Espana A, Sanchez-Carpintero I, Iraburu MJ, Lopez-Zabalza MJ: Pemphigus vulgaris autoantibodies induce apoptosis in HaCaT keratinocytes. *FEBS Lett* 2004;566:6–10.
- 45 Gniadecki R, Jemec GB, Thomsen BM, Hansen M: Relationship between keratinocyte adhesion and death: anoikis in acantholytic diseases. *Arch Dermatol Res* 1998;290:528–532.
- 46 Amagai M, Klaus-Kovtun V, Stanley JR: Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. *Cell* 1991;67:869–877.
- 47 Eyre RW, Stanley JR: Human autoantibodies against a desmosomal protein complex with a calcium-sensitive epitope are characteristic of pemphigus foliaceus patients. *J Exp Med* 1987;165:1719–1724.
- 48 Eyre RW, Stanley JR: Identification of pemphigus vulgaris antigen extracted from normal human epidermis and comparison with pemphigus foliaceus antigen. *J Clin Invest* 1988;81:807–812.
- 49 Waschke J, Bruggeman P, Baumgartner W, Zillikens D, Drenckhahn D: Pemphigus foliaceus IgG causes dissociation of desmoglein 1-containing junctions without blocking desmoglein 1 transinteraction. *J Clin Invest* 2005;115:3157–3165.
- 50 Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, Giudice GJ: A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. *J Clin Invest* 1993;92:2480–2488.
- 51 Giudice GJ, Liu Z, Diaz LA: An animal model of bullous pemphigoid: what can it teach us? *Proc Assoc Am Physicians* 1995;107:237–241.
- 52 Giudice GJ, Emery DJ, Zelickson BD, Anhalt GJ, Liu Z, Diaz LA: Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. *J Immunol* 1993;151:5742–5750.
- 53 Liu Z, Diaz LA, Swartz SJ, Troy JL, Fairley JA, Giudice GJ: Molecular mapping of a pathogenically relevant BP180 epitope associated with experimentally induced murine bullous pemphigoid. *J Immunol* 1995;155:5449–5454.

- 54 Liu Z, Giudice GJ, Swartz SJ, Fairley JA, Till GO, Troy JL, Diaz LA: The role of complement in experimental bullous pemphigoid. *J Clin Invest* 1995;95:1539–1544.
- 55 Chen R, Ning G, Zhao ML, Fleming MG, Diaz LA, Werb Z, Liu Z: Mast cells play a key role in neutrophil recruitment in experimental bullous pemphigoid. *J Clin Invest* 2001;108:1151–1158.
- 56 Liu Z, Giudice GJ, Zhou X, Swartz SJ, Troy JL, Fairley JA, Till GO, Diaz LA: A major role for neutrophils in experimental bullous pemphigoid. *J Clin Invest* 1997;100:1256–1263.
- 57 Hu P, Xiao H, Lin MS, Rubenstein DS, O’Keefe EJ, Jennette JC, Diaz LA, Liu Z: Myeloperoxidase-deficient mice are resistant to experimental bullous pemphigoid. *J Invest Dermatol* 2001;117:442.
- 58 Chen R, Fairley JA, Zhao ML, Giudice GJ, Zillikens D, Diaz LA, Liu Z: 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;169:3987–3992.
- 59 Liu Z, Shipley JM, Vu TH, Zhou X, Diaz LA, Werb Z, Senior RM: Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. *J Exp Med* 1998;188:475–482.
- 60 Liu Z, Shapiro SD, Zhou X, Twining SS, Senior RM, Giudice GJ, Fairley JA, Diaz LA: A critical role for neutrophil elastase in experimental bullous pemphigoid. *J Clin Invest* 2000;105:113–123.
- 61 Amagai M, Matsuyoshi N, Wang ZH, Andl C, Stanley JR: Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. *Nat Med* 2000;6:1275–1277.
- 62 Hanakawa Y, Schechter NM, Lin C, Garza L, Li H, Yamaguchi T, Fudaba Y, Nishifuji K, Sugai M, Amagai M, et al: Molecular mechanisms of blister formation in bullous impetigo and staphylococcal scalded skin syndrome. *J Clin Invest* 2002;110:53–60.
- 63 Amagai M, Yamaguchi T, Hanakawa Y, Nishifuji K, Sugai M, Stanley JR: Staphylococcal exfoliative toxin B specifically cleaves desmoglein 1. *J Invest Dermatol* 2002;118:845–850.
- 64 Mahoney MG, Wang ZH, Stanley JR: Pemphigus vulgaris and pemphigus foliaceus antibodies are pathogenic in plasminogen activator knockout mice. *J Invest Dermatol* 1999;113:22–25.
- 65 Mascaro JM Jr, Espana A, Liu Z, Ding X, Swartz SJ, Fairley JA, Diaz LA: Mechanisms of acantholysis in pemphigus vulgaris: role of IgG valence. *Clin Immunol Immunopathol* 1997;85:90–96.
- 66 Rock B, Labib RS, Diaz LA: Monovalent Fab’ immunoglobulin fragments from endemic pemphigus foliaceus autoantibodies reproduce the human disease in neonatal Balb/c mice. *J Clin Invest* 1990;85:296–299.
- 67 Payne AS, Ishii K, Kacir S, Lin C, Li H, Hanakawa Y, Tsunoda K, Amagai M, Stanley JR, Siegel DL: Genetic and functional characterization of human pemphigus vulgaris monoclonal autoantibodies isolated by phage display. *J Clin Invest* 2005;115:888–899.
- 68 Benndorf R, Hayess K, Ryazantsev S, Wieske M, Behlke J, Lutsch G: Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization-inhibiting activity. *J Biol Chem* 1994;269:20780–20784.
- 69 Geum D, Son GH, Kim K: Phosphorylation-dependent cellular localization and thermoprotective role of heat shock protein 25 in hippocampal progenitor cells. *J Biol Chem* 2002;277:19913–19921.
- 70 Panasenko OO, Kim MV, Marston SB, Gusev NB: Interaction of the small heat shock protein with molecular mass 25 kDa (hsp25) with actin. *Eur J Biochem* 2003;270:892–901.
- 71 Perng MD, Cairns L, van den IP, Prescott A, Hutcheson AM, Quinlan RA: Intermediate filament interactions can be altered by HSP27 and α B-crystallin. *J Cell Sci* 1999;112:2099–2112.
- 72 Evgrafov OV, Mersyanova I, Irobi J, van Den Bosch L, Dierick I, Leung CL, Schagina O, Verpoorten N, van Impe K, Fedotov V, et al: Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. *Nat Genet* 2004;36:602–606.
- 73 Lavoie JN, Hickey E, Weber LA, Landry J: Modulation of actin microfilament dynamics and fluid phase pinocytosis by phosphorylation of heat shock protein 27. *J Biol Chem* 1993;268:24210–24214.
- 74 Lavoie JN, Lambert H, Hickey E, Weber LA, Landry J: Modulation of cellular thermoresistance and actin filament stability accompanies phosphorylation-induced changes in the oligomeric structure of heat shock protein 27. *Mol Cell Biol* 1995;15:505–516.
- 75 Guay J, Lambert H, Gingras-Breton G, Lavoie JN, Huot J, Landry J: Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of heat shock protein 27. *J Cell Sci* 1997;110:357–368.

- 76 Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo AP, Kroemer G, Solary E, et al: Hsp27 negatively regulates cell death by interacting with cytochrome c. *Nat Cell Biol* 2000;2:645–652.
- 77 Berkowitz P, Hu P, Warren S, Liu Z, Diaz LA, Rubenstein DS: p38MAPK inhibition prevents disease in pemphigus vulgaris mice. *Proc Natl Acad Sci USA* 2006;103:12855–12860.
- 78 Rubenstein DS, Berkowitz P, Hu P, Liu Z, Diaz LA, Enghild JJ, Chua MP: Inhibition of p38MAPK prevents pemphigus vulgaris IgG induced cytoskeleton reorganization. *J Invest Dermatol* 2005;124:A28.
- 79 Waschke J, Spindler V, Bruggeman P, Zillikens D, Schmidt G, Drenckhahn D: Inhibition of Rho A activity causes pemphigus skin blistering. *J Cell Biol* 2006;175:721–727.
- 80 Lewis JE, Jensen PJ, Wheelock MJ: Cadherin function is required for human keratinocytes to assemble desmosomes and stratify in response to calcium. *J Invest Dermatol* 1994;102:870–877.
- 81 Lewis JE, Wahl JK 3rd, Sass KM, Jensen PJ, Johnson KR, Wheelock MJ: Cross-talk between adherens junctions and desmosomes depends on plakoglobin. *J Cell Biol* 1997;136:919–934.
- 82 Hu P, O’Keefe EJ, Rubenstein DS: Tyrosine phosphorylation of human keratinocyte β -catenin and plakoglobin reversibly regulates their binding to E-cadherin and α -catenin. *J Invest Dermatol* 2001;117:1059–1067.
- 83 Hu P, Berkowitz P, O’Keefe EJ, Rubenstein DS: Keratinocyte adherens junctions initiate nuclear signaling by translocation of plakoglobin from the membrane to the nucleus. *J Invest Dermatol* 2003;121:242–251.
- 84 Hu P, Berkowitz P, Madden VJ, Rubenstein DS: Stabilization of plakoglobin and enhanced keratinocyte cell-cell adhesion by intracellular O-glycosylation. *J Biol Chem* 2006;281:12786–12791.

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Autoimmunity to Type VII Collagen: Epidermolysis Bullosa Acquisita

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Abstract

Epidermolysis bullosa acquisita (EBA) is an acquired, mechanobullous disease characterized by autoimmunity to type VII collagen. Type VII collagen makes anchoring fibrils, structures that connect the epidermis and its underlying basement membrane zone to the papillary dermis. EBA patients exhibit skin fragility, blisters, scars and milia formation reminiscent of genetic dystrophic epidermolysis bullosa (DEB). DEB patients have diminutive or absent anchoring fibrils due to a genetic defect in the gene encoding type VII collagen. EBA patients have a decrease in normally functioning anchoring fibrils secondary to an abnormality in their immune system in which they produce 'pathogenic' IgG anti-type VII collagen antibodies. The pathogenicity of these autoantibodies has been demonstrated by passive transfer animal models, in which anti-type VII collagen antibodies injected into a mouse produced an EBA-like blistering disease in the animal. EBA has several distinct clinical presentations. It can present with features similar to DEB, bullous pemphigoid, cicatricial pemphigoid, Brunsting-Perry pemphigoid or IgA bullous dermatosis. Treatment for EBA is unsatisfactory, however, some therapeutic success has been reported with colchicine, dapsone, photophoresis, infliximab and intravenous immunoglobulin.

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Epidermolysis bullosa acquisita (EBA) is a prototypic autoimmune disease in which the autoantigen (self protein) and corresponding autoantibody are identified and well characterized [1, 2]. In EBA, the human autoantibody from afflicted patients can be injected into an animal, which will then develop an EBA-like disease [3]. This is in distinction from other presumed autoimmune diseases such as lupus erythematosus, scleroderma, mixed connective tissue disease, psoriasis and dermatomyositis.

EBA is a chronic, subepidermal blistering disease associated with autoimmunity to type VII collagen within anchoring fibrils located at the dermal-epidermal

junction. Roenigk et al. [4] defined the first diagnostic criteria for EBA: (1) negative family and personal history for bullous disease; (2) adult onset of the eruption; (3) spontaneous or trauma-induced blisters resembling those of hereditary dystrophic epidermolysis bullosa (DEB); (4) exclusion of all other bullous diseases. Subsequently, Kushniruk et al. [5] demonstrated IgG deposits at the dermal-epidermal junction of EBA patients.

Clinical Manifestations

Although the clinical spectrum of EBA is still being defined, the five following clinical variants have been established: (1) a ‘classical’ presentation; (2) a bullous pemphigoid (BP)-like presentation; (3) a cicatricial pemphigoid (CP)-like presentation; (4) a presentation reminiscent of Brunsting-Perry pemphigoid with scarring lesions predominantly localized to the head and neck; (5) a presentation similar to linear IgA bullous dermatosis (LABD) or chronic bullous disease of childhood.

Classical Presentation

The classical form of EBA is a mechanobullous disease marked by skin fragility and noninflammatory bullous lesions in an acral distribution. Mild classical EBA is reminiscent of porphyria cutanea tarda, whereas a more severe presentation approximates the hereditary form of recessive DEB [4]. Patients present with erosions, blisters and scars over trauma-prone surfaces such as the dorsal hands, knuckles, elbows, knees, sacral area and toes. The lesions heal with scarring and the formation of pearl-like milia cysts. Additionally, a scarring alopecia and some degree of nail dystrophy may occur. Patients with severe disease may have many of the same sequelae as those with hereditary forms of recessive DEB, such as scarring, loss of hair on the scalp, nail loss, fibrosis of the hands and fingers, and esophageal stenosis [6].

BP-Like Presentation

A second clinical presentation of EBA is a widespread, inflammatory vesiculobullous eruption involving the extremities, trunk, central body and skin folds [7]. The bullous lesions are tense and surrounded by inflamed or urticarial skin. Large areas of inflamed skin may be seen with only erythema or urticarial plaques.

CP-Like Presentation

EBA presenting with a mucosal predominant clinical appearance is reminiscent of CP [8]. These patients have erosions and scars on the mucosal surfaces of the mouth, upper esophagus, conjunctiva, anus or vagina.

Brunsting-Perry Pemphigoid-Like Presentation

Brunsting-Perry CP is a chronic, recurrent vesiculobullous eruption localized to the head and neck. Patients have residual scars, subepidermal bullae, minimal or no mucosa involvement and IgG deposits at the dermal-epidermal junction. A group of EBA patients with IgG autoantibodies directed to anchoring fibrils below the lamina densa has been reported with this constellation of findings. Thus, patients may present with the clinical phenotype of Brunsting-Perry pemphigoid, but have autoimmunity to type VII collagen [9].

LABD-Like Presentation

This form of EBA resembles LABD, being characterized by tense vesicles on bilateral extremities and mucous membrane involvement. Subepidermal blisters and a neutrophilic infiltrate are seen on routine histological examination and direct immunofluorescence (DIF) reveals linear IgA deposits at the basement membrane zone (BMZ) [10–13].

Childhood EBA is a rare disease with variable presentation. A review of 14 patients with childhood EBA reported that 5 presented as a LABD-like disease, 5 as BP-like and 4 as the classical type [14]. Eleven of fourteen had mucosal involvement and all patients had IgG deposits at the BMZ by DIF, in addition to other immunoreactants. Indirect immunofluorescence (IIF) was positive in 10 of 14 patient sera with predominantly IgG class antibodies. Although severe mucosal involvement is common in childhood EBA, the overall prognosis and response to treatment are more favorable than in adult EBA [14, 15].

EBA may be associated with various systemic diseases, such as inflammatory bowel disease, systemic lupus erythematosus (SLE), amyloidosis, thyroiditis, multiple endocrinopathy syndrome, rheumatoid arthritis, pulmonary fibrosis, chronic lymphocytic leukemia, thymoma and diabetes [4, 16]. Considering the combined experience of following over 60 patients from the University of North Carolina, Stanford University and Northwestern University, it appears that inflammatory bowel disease is the most frequent systemic disease associated with EBA [17].

Etiology and Pathogenesis

EBA is not a genetic disease with a Mendelian inheritance pattern, however, some EBA patients may have a genetic predisposition to autoimmunity [18]. For example, black patients in the southeastern United States with either EBA or bullous SLE have a high incidence of the HLA-DR2 phenotype. The calculated relative risk for EBA in black, HLA-DR2-positive individuals is 13.1, suggesting that this allele is involved with autoimmunity to anchoring

fibril collagen or is a marker for another gene existing in linkage disequilibrium with it. These results also imply that EBA and bullous SLE are immunogenetically related [18].

Although the etiology of EBA is unknown, evidence suggests an autoimmune pathogenesis. EBA antibodies bind to type VII collagen within anchoring fibrils and are detected by DIF of perilesional skin biopsies as IgG deposits at the dermal-epidermal junction [2, 19–21]. Anchoring fibrils are wheat stack-like structures that emanate perpendicularly from the lamina densa. They function to anchor the epidermis and its underlying BMZ to the papillary dermis [22, 23].

Patients with EBA have decreased numbers of normal anchoring fibrils at their dermal-epidermal junction, which is thought to be caused by IgG autoantibodies binding to the type VII collagen α -chains [8, 24]. This paucity of anchoring fibrils results in skin fragility, subepidermal blisters, milia formation and scarring.

Type VII collagen is composed of three identical α -chains, each consisting of a 145-kDa central collagenous triple-helical segment characterized by repeating Gly-X-Y amino acid sequences. The triple-helical segment is flanked by a large 145-kDa amino-terminal noncollagenous domain (NC1) and a small 34-kDa carboxyl-terminal noncollagenous domain (NC2) [23]. Within the extracellular space, type VII collagen molecules form antiparallel, tail-to-tail dimers stabilized by disulfide bonding through a small carboxyl-terminal NC2 overlap. After a portion of the NC2 domain is proteolytically removed, the antiparallel dimers aggregate laterally to form anchoring fibrils with large globular NC1 domains at both ends of the structure [25]. Sequence analysis of the NC1 domain revealed multiple submodules with homology to adhesive proteins [26]. These include a segment with homology to cartilage matrix protein, nine consecutive fibronectin type III-like repeats, and a segment with homology to the A domain of von Willebrand factor. Therefore, the NC1 domain may facilitate binding of type VII collagen to other BMZ and extracellular matrix components in order to stabilize the adhesion of the BMZ to the underlying dermis. Our study using recombinant NC1 demonstrated that NC1 interacts with various extracellular matrix components, including fibronectin, laminin-5, type I collagen and type IV collagen [27].

The major antigenic epitopes of type VII collagen are located within the NC1 domain [28–30]. However, a milder form of EBA was defined in children with tissue-bound and circulating autoantibodies directed against the central triple-helical domain of type VII collagen [15, 31]. Another group of patients was identified whose autoantibodies recognize epitopes in both the NC1 and the triple-helical domain [15]. Therefore, EBA exhibits a broader spectrum of autoantibody reactivities than previously assumed, which is an important consideration in designing laboratory tests for EBA autoantibodies. Epitope specificity

of EBA autoantibodies does not appear to correlate with clinicopathologic types, complement-fixing abilities or IgG subclasses.

The pathway leading to a reduction in the number of anchoring fibrils in lesional and perilesional skin of EBA patients and subsequent blister formation is unknown. It is possible that autoantibody deposition on the NC1 domain of anchoring fibrils compromises the function of type VII collagen by disturbing its interactions with other basement membrane or upper dermal components, such as type IV collagen, laminin-5 and fibronectin [28, 32]. Alternatively, EBA autoantibodies might interfere with the antiparallel dimer formation of type VII collagen and consequently anchoring fibril assembly [33]. These mechanisms attribute the skin fragility and noninflammatory blisters in patients with the classical EBA phenotype to defective lamina densa-dermal adherence. Another possibility involves antibody-induced complement fixation, leading to inflammation and tissue damage at the dermal-epidermal junction and subsequent blister formation. This mechanism likely predominates in the inflammatory, BP-like variety of EBA characterized by vesiculobullous lesions on inflamed skin [7].

An autoimmune etiology of EBA is supported by several independent lines of evidence derived from clinical, histologic and immunologic studies. Passive transfer animal models of EBA provide direct proof of the pathogenicity of EBA autoantibodies. We immunized rabbits and raised high-titer antiserum to the NC1 domain of human type VII collagen. Intradermal injection of these antibodies into hairless immunocompetent mice resulted in a skin condition and clinical picture similar to human EBA [26]. Specifically, the mice developed subepidermal blisters and nail dystrophy, and had circulating NC1 antibodies in their sera. Histologic examination revealed anti-NC1 IgG and murine complement deposits at their dermal-epidermal junction [34]. Sitaru et al. [35] obtained similar results when injecting mice with rabbit polyclonal antibodies to the NC1 domain of mouse type VII collagen. Furthermore, we recently generated a blistering disease with clinical, immunohistologic and ultrastructural features akin to human EBA by injecting mice with human EBA autoantibodies affinity-purified against an NC1 column [3]. These passive transfer experiments and the observations with bullous SLE suggest a key pathogenic role for EBA autoantibodies in epidermal-dermal separation and disease induction.

Laboratory Evaluation

Pathology

Routine histologic examination of lesional skin from EBA patients shows a subepidermal blister with clean separation between the epidermis and dermis.

The level of lesional inflammation observed by the clinician usually correlates well with the degree of inflammatory infiltrate seen within the dermis. Lesions reminiscent of recessive DEB or porphyria cutanea tarda are marked by a scarcity of inflammatory cells within the dermis [36]. BP-like lesions usually have significantly more inflammatory cells within the dermis, notably a mixture of lymphocytes, monocytes, neutrophils and eosinophils, and the histology may be difficult to distinguish from BP itself. Ultrastructural studies of EBA skin demonstrate a paucity of anchoring fibrils and an amorphous, electron-dense band just beneath the lamina densa [37].

Immunofluorescence

EBA patients have IgG deposits within the dermal-epidermal junction of their skin that are best observed by DIF of a biopsy specimen obtained from perilesional skin [18, 21]. Perilesional skin incubated in cold 1 M NaCl is fractured through the dermal-epidermal junction. This places the BP antigen (and any associated immune deposits) on the epidermal roof and the EBA antigen (and any associated immune deposits) on the dermal floor of the separation. In EBA patients, immune deposits are detected on the dermal side of the separation by a routine DIF method employing fluorescein-conjugated anti-human IgG. IgG is the predominant immunoglobulin subclass, but deposits of complement, IgA, IgM, factor B and properdin may also be detected.

Circulating autoantibodies can be detected by IIF of the patient's serum on a substrate of monkey or rabbit esophagus or human skin. These antibodies will stain the dermal-epidermal junction in a linear fashion that may be indistinguishable from BP sera. However, salt-split skin can be used to distinguish EBA and BP sera [38]. When human skin is incubated in 1 M NaCl, the dermal-epidermal junction fractures through the lamina lucida zone and places the BP antigen on the epidermal side of the split and all other BMZ components on the dermal side [39]. If the antibody labels the epidermal roof of the salt-split substrate, the patient does not have EBA and BP should be considered. In EBA or bullous SLE, the antibody labels the dermal side of the separation, however, bullous SLE can be ruled out by other serology and clinical criteria.

Table 1 summarizes the subepidermal bullous diseases that exhibit dermal fluorescence on salt-split skin immunofluorescence. Formerly, it was thought that only EBA and bullous SLE gave dermal labeling on immunofluorescence of salt-split skin, indicating the presence of immune deposits within the lamina densa or sublamina densa space. Conversely, epidermal roof labeling localized immune deposits within hemidesmosomes or the lamina lucida space. This notion was challenged by data from a subset of CP patients who have IgG autoantibodies against laminin-5 (a noncollagenous component of anchoring

Table 1. Sub-epidermal autoimmune bullous diseases with dermal staining via salt-split direct or indirect immunofluorescence

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1. Epidermolysis Bullosa Acquisita
 2. Bullous SLE
 3. Anti-Epiligrin (laminin-5) Cicatricial Pemphigoid
 3. “Chan’s Disease” – a.k.a. Protein 105 Pemphigoid
 4. “Zilliken’s Disease” – a.k.a. Protein 200 Pemphigoid
 5. “Ghohestani’s Disease” – a.k.a. Anti-type IV Collagen Bullous Disease
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filaments within the lamina lucida) that labeled the dermal floor of salt-split human skin by IIF and showed a dermal floor pattern by DIF. In addition, these patients had IgG immune deposits in the lower lamina lucida [40]. This discrepancy was explained by Ceilley et al. [41], who showed that laminin-5 remains on the dermal side when the BMZ is fractured by salt. Finally, a third subepidermal blistering disease that gives dermal staining by salt-split skin immunofluorescence was reported by Ghohestani et al. [42]. This disease is associated with renal insufficiency and patients have IgG autoantibodies directed against the $\alpha 5$ -chain of type IV collagen, a component of the lamina densa zone of the BMZ.

Immunoelectron Microscopy

The gold standard for the diagnosis of EBA is the localization of immune deposits within the dermal-epidermal junction of their skin by immunoelectron microscopy. Neiboer et al. [20] and Yaoita et al. [21] demonstrated that patients with EBA have immune deposits within the lamina densa and sublamina densa zones of the cutaneous BMZ. This is distinct from the deposits in BP, which are located within the hemidesmosome area.

Western Immunoblotting

Antibodies in EBA sera bind to a 290-kDa band, corresponding to the α -chain of type VII collagen, in Western blots of human skin basement membrane proteins [1]. Sera from all other primary blistering diseases will not bind to type VII collagen. Often a second band of 145 kDa will also be present. This band is the carbohydrate-rich amino-terminal globular NC1 domain of the type VII collagen α -chain. NC1 contains the antigenic epitopes of EBA autoantibodies, bullous SLE autoantibodies and monoclonal antibodies against type VII collagen [1, 2, 28, 29].

Enzyme-Linked Immunosorbent Assay

Chen and coworkers [3, 27, 43] developed an enzyme-linked immunosorbent assay (ELISA) utilizing recombinant purified posttranslationally modified NC1 for autoantibody detection in EBA and bullous SLE patients. This new ELISA is more sensitive than immunofluorescence and Western blotting and is highly specific for anti-type VII collagen antibodies.

Treatment

All EBA patients require supportive therapy, including instruction in wound care and strategies for avoiding trauma. In some patients, the use of sunscreens and the avoidance of prolonged sun exposure may be helpful in preventing new lesions on the dorsal hands and knuckles.

There are three independent reports of EBA patients responding to high doses of colchicine, which is often used as a first-line drug [44]. Colchicine is a well-known microtubule inhibitor that also appears to downregulate autoimmunity by inhibition of antigen presentation to T cells [45]. Its side effect profile is relatively benign compared with other therapeutic choices. However, diarrhea may make it difficult for many patients to achieve a dose high enough to control the disease. Moreover, because of this side effect, the authors are reluctant to try colchicine in EBA patients who have concomitant inflammatory bowel disease [45].

Cyclosporin, an immunosuppressive agent, has also proved beneficial in EBA patients [46, 47]. However, this drug should only be used as a last-resort measure due to its long-term toxicity.

Classical noninflammatory mechanobullous EBA patients are usually refractory to high doses of systemic glucocorticoids, azathioprine, methotrexate and cyclophosphamide. However, these drugs may be helpful in controlling EBA in patients with the inflammatory BP-like presentation. Some patients respond to dapsone, especially when neutrophils are present in their dermal infiltrate.

Photophoresis has been used in Sézary syndrome, mycosis fungoides and a variety of autoimmune bullous diseases [48]. This procedure may lead to improved dermal-epidermal adherence in EBA patients, as measured by remarkably lengthened suction blistering times and improved clinical parameters [49]. In one EBA patient, photophoresis had a dramatic effect in a life-threatening situation [50].

Lastly, intravenous immunoglobulin has been shown to be helpful in a variety of autoimmune bullous dermatoses, particularly pemphigus [51]. Intravenous immunoglobulin has reportedly been effective in some EBA patients, although it is unknown how γ -globulin may invoke clinical improvement [52].

References

- 1 Woodley DT, Briggaman RA, O'Keefe EJ, Inman AO, Queen LL, Gammon WR: Identification of the skin basement membrane autoantigen in epidermolysis bullosa acquisita. *N Engl J Med* 1984;310:1007–1013.
- 2 Woodley DT, Burgeson RE, Lunstrum G, Bruckner-Tuderman L, Reese MJ, Briggaman RA: The epidermolysis bullosa acquisita antigen is the globular carboxyl terminus of type VII procollagen. *J Clin Invest* 1988;81:683–687.
- 3 Woodley DT, Ram R, Doostan A, Bandyopadhyay P, Huang Y, Remington J, Hou YP, Keene DR, Liu Z, Chen M: Induction of epidermolysis bullosa acquisita in mice by passive transfer of autoantibodies from patients. *J Invest Dermatol* 2006;126:1324–1330.
- 4 Roenigk HH Jr, Ryan JG, Bergfeld WF: Epidermolysis bullosa acquisita: report of three cases and review of all published cases. *Arch Dermatol* 1971;103:1–10.
- 5 Kushniruk W: The immunopathology of epidermolysis bullosa acquisita. *Can Med Assoc J* 1973;108:1143–1146.
- 6 Stewart MI, Woodley DT, Briggaman RA: Acquired epidermolysis bullosa and associated symptomatic esophageal webs. *Arch Dermatol* 1991;127:373–377.
- 7 Gammon WR, Briggaman RA, Woodley DT, Heald PW, Wheeler CE Jr: Epidermolysis bullosa acquisita: a pemphigoid-like disease. *J Am Acad Dermatol* 1984;11:820–832.
- 8 Dahl MGC: Epidermolysis bullosa acquisita: a sign of cicatricial pemphigoid? *Br J Dermatol* 1979;101:475–484.
- 9 Kurzhals G, Stolz W, Meurer M, Kunze J, Braun-Falco O, Krieg T: Acquired epidermolysis bullosa with the clinical features of Brunsting-Perry cicatricial bullous pemphigoid. *Arch Dermatol* 1991;127:391–395.
- 10 Hashimoto T, Ishiko A, Shimizu H, Tanaka T, Dodd HJ, Bhogal BS, Black MM, Nishikawa T: A case of linear IgA bullous dermatosis with IgA anti-type VII collagen autoantibodies. *Br J Dermatol* 1996;134:336–339.
- 11 Rusenko KW, Gammon WR, Briggaman RA: Type VII collagen is the antigen recognized by IgA anti-sub lamina densa autoantibodies. *J Invest Dermatol* 1989;92:510.
- 12 Bauer JW, Schaeppi H, Metz D, Muss W, Pohla-Gubo G, Hametner R, Ruckhofer J, Grabner G, Hinter H: Ocular involvement in IgA-epidermolysis bullosa acquisita. *Br J Dermatol* 1999;141:887–892.
- 13 Lee CW: Serum IgA autoantibodies in patients with epidermolysis bullosa acquisita: a high frequency of detection. *Dermatology* 2000;200:83–84.
- 14 Edwards S, Wakelin SH, Wojnarowska F, Marsden RA, Kirtschig G, Bhogal B, Black MM: Bullous pemphigoid and epidermolysis bullosa acquisita: presentation, prognosis and immunopathology in 11 children. *Pediatr Dermatol* 1998;15:184–190.
- 15 Callot-Mellot C, Bodemer C, Caux F, Bourgault-Villada I, Fraitag S, Goudie G, Heller M, de Prost Y, Prost C: Epidermolysis bullosa acquisita in childhood. *Arch Dermatol* 1997;133:1122–1126.
- 16 Burke WA, Briggaman RA, Gammon WR: Epidermolysis bullosa acquisita in a patient with multiple endocrinopathies syndrome. *Arch Dermatol* 1986;122:187–189.
- 17 Chan L, Woodley DT: Pemphigoid: bullous and cicatricial; in Lichtenstein LM, Fauci AS (eds): *Current Therapy in Allergy, Immunology and Rheumatology*, ed 5. St. Louis, Mosby, 1996, p 93.
- 18 Gammon WR, Heise ER, Burke WA, Fine JD, Woodley DT, Briggaman RA: Increased frequency of HLA DR2 in patients with autoantibodies to EBA antigen: evidence that the expression of autoimmunity to type VII collagen is HLA class II allele associated. *J Invest Dermatol* 1988;91:228–232.
- 19 Gibbs RB, Minus HR: Epidermolysis bullosa acquisita with electron microscopical studies. *Arch Dermatol* 1975;111:215–220.
- 20 Nieboer C, Boersma DM, Woerdeman MJ, Kalsbeek GL: Epidermolysis bullosa acquisita: immunofluorescence, electron microscopic and immunoelectron microscopic studies in four patients. *Br J Dermatol* 1980;102:383–392.

- 21 Yaoita H, Briggaman RA, Lawley TJ, Provost TT, Katz SI: Epidermolysis bullosa acquisita: ultrastructural and immunological studies. *J Invest Dermatol* 1981;76:288–292.
- 22 Briggaman RA, Wheeler CE Jr: The epidermal-dermal junction. *J Invest Dermatol* 1975;65:71–84.
- 23 Keene DR, Sakai LY, Lunstrum GP, Morris NP, Burgeson RE: Type VII collagen forms an extended network of anchoring fibrils. *J Cell Biol* 1987;104:611–621.
- 24 Ray TL, Levine JB, Weiss W, Ward PA: Epidermolysis bullosa acquisita and inflammatory bowel disease. *J Am Acad Dermatol* 1982;6:242–252.
- 25 Bruckner-Tuderman L, Nilssen O, Zimmermann DR, Dours-Zimmermann MT, Kalinke DU, Gedde-Dahl T Jr, Winberg JO: Immunohistochemical and mutation analyzes demonstrates that procollagen VII is processed to collagen VII through removal of the NC-2 domain. *J Cell Biol* 1995;131:551–559.
- 26 Christiano AM, Greenspan DS, Lee S, Uitto J: Cloning of human type VII collagen: complete primary sequence of the $\alpha 1$ (VII) chain and identification of intragenic polymorphisms. *J Biol Chem* 1994;269:20256–20262.
- 27 Chen M, Marinkovich MP, Veis A, Cai X, Rao CN, O'Toole EA, Woodley DT: Interactions of the amino-terminal noncollagenous (NC1) domain of type VII collagen with extracellular matrix components. *J Biol Chem* 1997;272:14516–14522.
- 28 Lapiere J-C, Woodley DT, Parente MG, Iwasaki T, Wynn KC, Christiano AM, Uitto J: Epitope mapping of type VII collagen: identification of discrete peptide sequences recognized by sera from patients with acquired epidermolysis bullosa. *J Clin Invest* 1993;92:1831–1839.
- 29 Jones DA, Hunt SW 3rd, Prisanh PS, Briggaman RA, Gammon WR: Immunodominant autoepitopes of type VII collagen are short, paired peptide sequences within the fibronectin type III homology region of the noncollagenous (NC1) domain. *J Invest Dermatol* 1995;104:231–235.
- 30 Woodley DT, O'Keefe EJ, McDonald JA, Reese MJ, Briggaman RA, Gammon WR: Specific affinity between fibronectin and the epidermolysis bullosa acquisita antigen. *J Clin Invest* 1987;79:1826–1830.
- 31 Park SB, Cho KH, Youn JL, Hwang DH, Kim SC, Chung JH: Epidermolysis bullosa acquisita in childhood: a case mimicking chronic bullous dermatosis of childhood. *Clin Exp Dermatol* 1997;22:220–222.
- 32 Lapiere J-C, Chen JD, Iwasaki T, Hu L, Uitto J, Woodley DT: Type VII collagen specifically binds fibronectin via a unique subdomain within the collagenous triple helix. *J Invest Dermatol* 1994;103:637–641.
- 33 Chen M, Keene DR, Costa FK, Tahk SH, Woodley DT: The carboxyl terminus of type VII collagen mediates antiparallel dimer formation and constitutes a new antigenic epitope for epidermolysis bullosa aquisita autoantibodies. *J Biol Chem* 2001;276:21649–21655.
- 34 Woodley DT, Chang C, Saadat P, Ram R, Liu Z, Chen M: Evidence that anti-type VII collagen antibodies are pathogenic and responsible for the clinical, histological, and immunological features of epidermolysis bullosa acquisita. *J Invest Dermatol* 2005;124:958–964.
- 35 Sitaru C, Mihai S, Otto C, Chiriac MT, Hausser I, Dotterweich B, Saito H, Rose C, Ishiko A, Zillikens D: Induction of dermal-epidermal separation in mice by passive transfer of antibodies specific to type VII collagen. *J Clin Invest* 2005;115:870–878.
- 36 Lever WF, Schaumburg-Lever G: Congenital diseases (genodermatoses); in Lever WF, Schaumburg-Lever G (eds): *Histopathology of the Skin*. Philadelphia, Lippincott, 1990, p 78.
- 37 Richter BJ, McNutt NS: The spectrum of epidermolysis bullosa acquisita. *Arch Dermatol* 1979;115:1325–1328.
- 38 Gammon WR, Kowalewski C, Chorzelski TP, Kumar V, Briggaman RA, Beutner EH: Direct immunofluorescence studies of sodium chloride: separated skin in the differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita. *J Am Acad Dermatol* 1990;22:664–670.
- 39 Woodley DT, Sauder D, Talley MJ, Silver M, Grotendorst G, Qwarnstrom E: Localization of basement membrane components after dermal-epidermal junction separation. *J Invest Dermatol* 1983;81:149–153.
- 40 Domloge-Hultsch N, Anhalt GJ, Gammon WR, Lazarova Z, Briggaman R, Welch M, Jabs DA, Huff C, Yancey KB: Antiepilgrin cicatricial pemphigoid: a subepithelial bullous disorder. *Arch Dermatol* 1994;130:1521–1529.

- 41 Ceilley E, Watanabe N, Shapiro D, Verrando P, Bauer EA, Burgeson R, Briggaman RA, Woodley DT: Labeling of fractured human skin with antibodies to BM 600/nicein, epiligrin, kalinin and other matrix components. *J Dermatol Sci* 1993;5:97–103.
- 42 Ghohestani RF, Hudson BG, Claudy A, Uitto J: The $\alpha 5$ chain of type IV collagen is the target of IgG autoantibodies in a novel autoimmune disease with subepidermal blisters and renal insufficiency. *J Biol Chem* 2000;275:16002–16006.
- 43 Chen M, Chan LS, Cai X, O'Toole EA, Sample JC, Woodley DT: Development of an ELISA for rapid detection of anti-type VII collagen autoantibodies in epidermolysis bullosa acquisita. *J Invest Dermatol* 1997;108:68–72.
- 44 Cunningham BB, Kirchmann TT, Woodley D: Colchicine for epidermolysis bullosa (EBA). *J Am Acad Dermatol* 1996;34:781–784.
- 45 Mekori YA, Chowers Y, Ducker I, Klajman A: Inhibition of delayed hypersensitivity reaction by colchicine. II. Colchicine inhibits interferon- γ induced expression of HLA-DR on gut epithelial cell line. *Clin Exp Immunol* 1989;78:230–232.
- 46 Crow LL, Finkle JP, Gammon WR, Woodley DT: Clearing of epidermolysis bullosa acquisita on cyclosporine A. *J Am Dermatol* 1988;19:937–942.
- 47 Connolly SM, Sander HM: Treatment of epidermolysis bullosa acquisita with cyclosporine. *J Am Dermatol* 1987;16:890.
- 48 Rook AH, Jegasothy BV, Heald P, Nahass GT, Ditre C, Witmer WK, Lazarus GS, Edelson RL: Extracorporeal photochemotherapy for drug-resistant pemphigus vulgaris. *Ann Intern Med* 1990;112:303–305.
- 49 Miller JL, Stricklin GP, Fine JD, King LE, Arzubiaga MC, Ellis DL: Remission of severe epidermolysis bullosa acquisita induced by extracorporeal photochemotherapy. *Br J Dermatol* 1995;133:467.
- 50 Gordon K, Chan LS, Woodley DT: Treatment of refractory epidermolysis bullosa acquisita with extracorporeal photochemotherapy. *Br J Dermatol* 1997;136:415–420.
- 51 Bystryn J-C, Jiao D, Natow S: Treatment of pemphigus with intravenous immunoglobulin. *J Am Acad Dermatol* 2002;47:358–363.
- 52 Meier F, Sönnichsen K, Schaumburg-Lever G, Dopfer R, Rassner G: Epidermolysis bullosa acquisita: efficacy of high-dose intravenous immunoglobulins. *J Am Acad Dermatol* 1993;29:334–337.

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Pathomechanisms of Lichen Planus Autoimmunity Elicited by Cross-Reactive T Cells

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Abstract

Lichen planus (LP) is an idiopathic inflammatory disease of the skin and mucous membranes, characterized by an autoimmune attack on the epidermis by skin-infiltrating T cells. It remains unknown, however, how such autoaggressive T cells could be activated in vivo to cause epidermal damage; we hypothesize that memory T cells specific for a previously encountered virus could cross-react with other antigens, including contact allergens, drugs and other heterologous viruses in the absence of cognate antigen, and cause epidermal damage. This hypothesis provides an explanation for an intimate relationship between exposure to a number of exogenous agents, such as viruses and drugs, and the development of LP. In addition to T cells migrating from the circulation, T cells indigenously residing in the epidermis, such as intraepidermal CD8⁺ T cells, would also be involved in tissue damage. This population is typically detected at high frequencies in the resting lesion of fixed drug eruption, which is a simplified disease model for LP. Fucosyltransferase VII, essential for generating E-selectin ligand, is shown to play an indispensable role in inducing the accumulation of relevant skin-homing T cells at sites of LP lesions; however, the alternative notion should be appreciated that T cell recruitment to the skin is also crucial for host defense and that T cells frequently found in LP lesions could display beneficial properties for the host.

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Most organ-specific autoimmune diseases are characterized by the selective targeting of a single organ or tissue by a certain population of autoreactive T cells. Thus, many of these diseases are classified according to what organs and tissues are targeted by the immune response [1]. It is well known that the healthy immune system contains potentially autoaggressive T cells as normal components. Indeed, we previously established a murine model for lichen planus (LP) by employing autoreactive T cells capable of producing interferon

(IFN)- γ and tumor necrosis factor- α (TNF- α) [2–4], which were originally isolated from healthy mice. Although many different mechanisms have been invoked, it is still not precisely clear how autoreactive T cells could be activated *in vivo* to cause tissue destruction. One possible mechanism is that environmental factors are required to activate the preexisting, potentially autoaggressive T cells in the periphery before the onset of an autoimmune disease. In this regard, there are frequent temporal associations between infections and the development of autoimmune diseases in mice and humans. Among various pathogens, there is now a large body of evidence implicating a virus in several different autoimmune diseases, including LP. Because recent studies have shown that memory T cells specific for a previously encountered virus can be cross-reactive with a heterologous virus [5, 6], these T cells would be expected to respond to other antigens, including drugs and other viruses, even in the absence of cognate antigen, and to cause tissue destruction. In this review, much emphasis has been placed on how memory T cells could be activated upon exposure to various insults, such as contact allergens, drugs and viruses.

Contact Allergens

Contact allergy to a variety of metals has been shown to be important in the pathogenesis of oral LP [7–9]. The metals that aggravate oral LP include amalgam (mercury), copper, palladium, beryllium and gold; mercury and mercury compounds appear to be the most common allergens in amalgam with the other metals being rarely responsible for allergic reactions [9]. Because involved allergens such as inorganic mercury are thought to be dissolved and spread via saliva and patients would therefore be sensitized with these allergens via mucosal surfaces, mucosal lesions can extend beyond the contact areas. Because positive patch test reactions to inorganic mercury compounds identify a subset of oral LP patients who have a high probability of benefiting from removal of amalgam dental restorations, we suggest that patch tests should be performed in patients with oral LP. Importantly, however, it should be noted that positive patch test reading may be delayed until days 10 or 17 in some patients [10]; this may explain the different reported frequencies of positive patch test reactions in the literature. Previous studies demonstrated that the vast majority of patients with oral LP showed improvement after the removal of amalgam fillings, regardless of patch test results to amalgam and other inorganic mercury compounds [9]. This may indicate that those patients who have negative patch test reactions but improve after the removal or replacement of amalgam fillings may be responding to the removal of additional factors such as ill-fitting dental

restoration and dental plaque, thereby reducing the element of continuous irritation and bacterial infections.

Viral Etiology

Earlier studies reported a high prevalence of chronic liver disease in patients with LP [11, 12]. These studies suggested that, when chronic liver disease is associated with LP, hepatitis C virus (HCV) is generally the cause of the liver disease. Indeed, higher prevalences of HCV infection were reported in patients with LP than in the corresponding general population in those areas where HCV is highly prevalent [13], while this was not the case in other areas; this association has been demonstrated in Japan, Italy, Spain and Iran, but not in the United Kingdom, France, the Netherlands, Germany and Scandinavia. No cases of HCV infection or liver abnormality were found in the United States. Thus, HCV could be the cause of some cases of LP in those populations most exposed to HCV infection [14]. The advent of polymerase chain reaction (PCR) encouraged the search for potentially relevant viruses in tissue. This technique is particularly useful when conventional laboratory methods, such as viral culture and serology, are unsatisfactory. The presence of HCV RNA in the oral LP lesions was subsequently confirmed with PCR technique [15], indicating a direct role of HCV in the development of these oral lesions. However, in some studies, HCV RNA was isolated from the oral mucosal tissue in patients with HCV, regardless of the presence of oral LP, not supporting a strong association with HCV infection. Thus, the attempt to detect HCV RNA and HCV-encoded proteins in LP lesions has given conflicting results. These findings indicate that oral LP may be only associated with a minority of HCV infections. Some reports described marginal significance toward an association between HCV and the reticular pattern of oral LP [16], while others reported a significant relationship between HCV and the erosive variant of oral LP [17]. Thus, there are no convincing data so far to support a direct pathogenetic role for HCV in LP, indicating that HCV alone is not sufficient for the development of LP. Possible explanations [18] for these conflicting data include: (1) the detection of HCV RNA could simply reflect the absorption of viral particles onto the cellular surface membrane via Fc receptor-mediated binding of antibody-coated viral particles; (2) there is a scarcity of active viral replication, as evidenced by the inability to constantly detect HCV genomic sequences in LP lesions of most patients with HCV-associated LP; (3) HCV itself may not be of pathologic importance in the skin and only represents a triggering factor necessary for immune system alterations; (4) an interaction with other factors (such as genetic, infectious and/or environmental agents) is required (for example, HCV

infection might only cause LP in patients with a predisposing HLA haplotype or those with coinfection with other viruses or an underlying susceptibility for autoimmune disease). So far, accumulating data suggest the third and/or fourth explanations. Possibly, the most relevant finding to suggest these explanations is increased frequency of HLA-DR6 allele in Italian patients with HCV-associated oral LP [19], suggesting that CD4⁺ T cells activated upon recognition of HCV-encoded peptides bound to HLA-DR6 molecules could be directly involved in the pathogenesis of LP. In support of this possible explanation, Campisi et al. [20] reported no case of oral LP in 74 human immunodeficiency virus (HIV)- and HCV-positive patients and 5 cases in 104 HIV-negative, HCV-positive patients, suggesting that the functional immunosuppression because of CD4 deficiency caused by HIV does not allow the triggering of cytotoxic mechanism leading to oral LP. Indeed, a recent tetramer analysis shows that HCV-specific CD4⁺ and/or CD8⁺ T cells are present at high frequencies in oral LP lesions compared with the circulating compartment and suggests that they are involved in these inflammatory lesions [21].

The recent introduction of combination therapeutic schemes including new and improved forms of established antiviral agents such as pegylated IFN- α and ribavirin that offers the chance of viral eradication could have significant effect on the rate of oral LP. However, recent studies have shown that no significant difference can be found in the rate of oral LP between untreated patients and those using these antiviral agents [20].

With regard to the role of other viruses in LP, human herpesvirus (HHV) 6 was detected in 67–100% of oral LP lesions by *in situ* hybridization and immunohistochemical techniques, while it was absent from normal oral tissues [22]. A recent retrospective survey of 18 lesional LP samples, as well as 11 non-lesional LP samples and 11 lesional psoriasis samples, showed that 11 of the 18 lesional samples had HHV-7 DNA, compared with 1 of the 11 nonlesional samples and 2 of the lesional psoriasis samples [23]. Immunohistochemical studies also showed that lesional LP dermis contained significantly more HHV-7⁺ cells than nonlesional LP, psoriatic or normal dermis [23]. Sporadic case reports also show that LP lesions developed in areas previously affected by recent herpes simplex virus (HSV) or varicella zoster virus infection [24], although the presence of these viruses in the lesions was not confirmed by PCR. Al-Mutairi et al. [25] also reported a case of LP, in whom HgCl₂ in the dental amalgam might have triggered an isotopic response at the healed herpes zoster sites; the patient had had herpes zoster affecting the same region 4 months before the appearance of LP lesions. Although many cutaneous reactions such as granuloma annulare are known to develop within resolved varicella zoster lesions [26], there are few reports of LP occurring in sites of resolved herpes zoster [25]. This may represent an immunological reaction to

minute amounts of viral proteins that persist even after the virus has been cleared from involved sites, because viral DNA was not necessarily detected in these LP lesions. However, considerable caution must be exercised in attempting to use these techniques to make conclusions about a role of the virus in etiology. Prompted by previous studies indicating the increased detection of human papillomavirus in oral LP lesions [27], Boyd et al. [28] sought to evaluate the presence of human papillomavirus DNA in LP lesions; however, more in-depth evaluation demonstrated specimen contamination. In addition, studies of ubiquitous viruses, such as Epstein-Barr virus (EBV), HHV-6 and HHV-7, are seriously hindered by the limited diagnostic value of conventional PCR analyses, which cannot differentiate between latent and active infection. Thus, the presence of viral DNA in the lesion cannot be taken as proof of causation of the disease, as viral DNA from a past infection might persist in the lesion and irrelevant viruses might enter the lesion as part of a systemic infection or by seeding from a site elsewhere in the body. Immunological and molecular techniques that allow direct detection of virus replication in *in vivo* and longitudinal studies of virus replication are therefore essential to establish a solid association between the virus and the disease. Thus, studies purporting to prove that a certain virus causes a given disease must be scrutinized with great caution.

A number of reports have described the appearance of LP after administration of different types of hepatitis B virus vaccines [29]. The time interval between the initial dose and the development of cutaneous or mucosal lesions has varied from a few days to 5 months. It is recommended that patients showing LP before completing the vaccination avoid further injections, because they are more likely to develop severe LP lesions.

How, then, can viral infection cause the disease? Is there clinical evidence that such viral infections linked to the induction of an autoimmune attack by T cells on the epidermis actually take place? Viral infection can induce or trigger autoimmune disease via at least two mechanisms. One potential mechanism is molecular mimicry [30, 31]. According to this theory, viruses possess antigenic determinants that can also be recognized by the host immune system. Evidence for such cross-reactive immune responses between viruses and self antigens has been demonstrated by many investigators [32, 33]; in fact, autoimmune keratitis can be induced by T cells specific for a peptide derived from HSV-1 in immunodeficient animals [33]. In support of this possibility, recent studies have shown that a single T cell receptor can recognize a broad range of epitopes, including peptides with totally different sequences [34, 35]. Another mechanism by which viruses can induce or trigger autoimmune disease is bystander activation [36]. In this theory, viral infections could stimulate innate immune responses, thereby upregulating costimulatory molecules and self antigen expression on antigen-presenting cells and releasing cytokines. Such events

would be initiated by type I IFN, mainly IFN- α , during viral infections. This sequence of events results in activation of otherwise dormant autoreactive T cells. Indeed, appearance or exacerbation of LP has been observed during treatment of hepatitis C or melanoma with IFN- α [37, 38]. Furthermore, a recent study provides evidence that supports a key role for IFN- α produced by plasmacytoid dendritic cells in chronic cytotoxic inflammation of LP by recruiting cytotoxic effector Th1 cells [39]. In view of the key function of type I IFN to enhance the cytotoxic functions of natural killer cells and the differentiation of virus-specific cytotoxic T cells, both of which recognize and eliminate virus-infected cells [40], these findings could be interpreted such that virus-specific T cells, although required for virus clearance, may be critical for the development of LP. This interpretation is consistent with the finding that HCV-specific CD4+ and/or CD8+ T cells are present with high frequencies in oral LP lesions [21]. Indeed, there is also data that CD4+ T cells that are positive for the cytotoxic effector molecules, perforin and granzyme, can mediate virus-specific immune control as effectors in their own right by directly killing infected cells [41]. Given the ability of CD4+ T cells to maintain protective CD8+ T cell immunity and to provide helper functions for antigen-presenting cells, CD4+ T cells would be more efficient at controlling EBV, cytomegalovirus (CMV) and other herpesviruses than CD8+ T cells. In support of this possibility, a recent immunohistochemical study of perforin expression in peripheral blood lymphocytes and skin-infiltrating cells in patients with LP has shown that upregulation of perforin expression in skin-infiltrating cells is much higher than that in peripheral blood, especially when CD4+ T cells are analyzed [42]. Also, in our mouse model for LP, the lichenoid tissue reaction (LTR) can be successfully induced in normal mice by local injection of CD4+ autoreactive T cells that can produce IFN- γ and TNF- α upon recognition of self major histocompatibility complex (MHC) class II antigens [2–4, 43]. These results indicate the importance of modulating the numbers and activity of antiviral CD4+ T cells responding to viral antigens without adversely affecting virus clearance.

Despite extensive search for the presence of viral genes in the LP lesions, PCR studies measuring viral genes within skin tissues or in blood have been quite inconclusive, although virus-specific T cells are present in the lesions; therefore, it seems that the pathology is a direct consequence of the immune response. The lack of detectable viral genes or viral antigen expression could simply be due to efficient removal of the productively infected cells by cytotoxic T cells, because the appearance of virus-specific cytotoxic T cells has been shown to coincide with a dramatic fall in viral load. However, several studies demonstrated that the herpesvirus (EBV, HSV, varicella zoster virus, HHV-6, HHV-7 and CMV) DNA can be readily and frequently detected in tissues despite the presence of virus-specific T cells at high frequencies. How and why

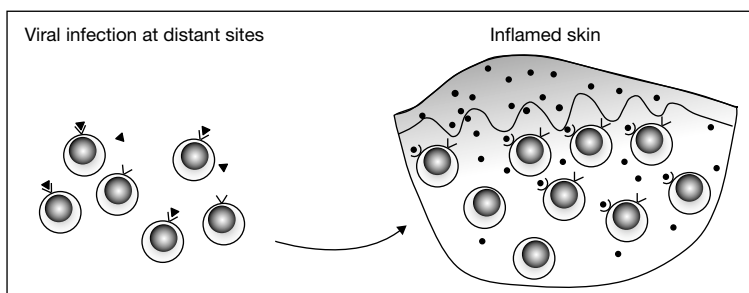


Fig. 1. At inflamed skin sites, nonspecific trapping of virus-specific T cells activated systemically or locally upon viral infection (closed triangles) and their subsequent activation in response to cross-reactive antigens (closed circles), such as contact allergens, drugs and other viruses in the absence of cognate antigen. These skin lesions would be recognized as LP lesions showing lesional onset or exacerbation upon exposure to contact allergens or drug intake.

then are virus-specific T cells recruited to and expanded in the lesions in the absence of viral genes and antigens? In view of the previous finding that virus-specific T cells are nonspecifically trapped within inflamed sites [44], a reasonable explanation is that virus-specific T cells that are activated systemically or locally at distant sites in response to the viral infection or reactivation would be trapped and expanded at inflamed skin sites and mediate tissue damage in the absence of their cognate antigen in the LP lesion (fig. 1). This possibility is supported by a recent study showing that virus-specific T cells generated in response to a viral infection restricted to sites outside the liver can trigger T cell-mediated collateral damage despite the absence of viral antigen in the liver [45]. This possibility has considerable clinical relevance, because there are many instances in which LP lesions often appear at the site where nonspecific insult such as trauma is supplied, known as Koebner phenomenon. In the light of a possible association with the class II HLA-DQ allele, we favor a primary role for virus-specific CD4⁺ T cells in the pathogenesis of LP. CD8⁺ T cells would also play a role in perpetuating chronic inflammation.

Drug Etiology

Eruptions similar or identical to LP clinically and histologically have been reported to occur following drug administration, often referred to as lichenoid drug eruptions (LDE). A wild variety of drugs have been associated with LDE

and the list of such drugs increases steadily [18], although recurrence of the lesions subsequent to rechallenge with the drug has not been documented for the majority of these drugs. In the majority of patients, LDE appeared to be diagnosed dependently on a temporal relationship between drug intake and onset of the lesions as well as resolution upon withdrawal of the drug. Recent studies have linked viral infections to the development of LP [15, 21–24], but it is not known whether there might be a link between viral infections and drug-induced exacerbation of the lesions. Is there clinical evidence that such viral infections linked to the subsequent induction of the LP or LDE actually take place?

We have recently experienced a patient with chronic hepatitis due to HCV, in which papulosquamous eruptions resembling lichenoid eruptions recurred in the same sites after IFN monotherapy for chronic hepatitis C. A 64-year-old woman presented with a 2-year history of erythematous scaling eruption: they began on her breasts and slowly spread to her neck, trunk, abdomen and thighs. Two years before her initial presentation, she had been started on natural IFN- β (6×10^6 U). Three months later, she noticed the asymptomatic macules on her breasts. Gradual worsening of the eruption was noted during the next 2 months. Because of the possibility of a drug eruption, the patient was asked not to use those drugs considered most likely to be causing the eruption. After 1 month there were no detectable lesions. However, the patient's course thereafter was complicated by some flares of erythematous macules in precisely the same sites on the trunk 3–4 weeks after she had started to ingest new medications. During several flares of erythematous macules, HCV RNA and alanine transaminase levels fluctuated considerably. However, as the flare recurring after starting new medications gradually subsided, HCV RNA levels inversely increased. Thus, there was good inverse correlation between flares of the plaques and HCV RNA levels. Flares of the plaques were frequently seen over a period of 3 years, during which time HCV RNA levels remained low; however, an increase in HCV RNA levels was temporally associated with a spontaneous resolution of the lesions, despite continuous administration of the drug which had caused the flare previously. These results suggest that a sustained anti-HCV immune response can cause immunopathology and that there is close inverse correlation between HCV viral load and the magnitude of clinical symptoms of drug eruption. In this patient, IFN would have represented a triggering factor that can stimulate otherwise silencing antiviral immune responses. The mechanisms by which anti-HCV immune responses are activated upon introduction of drugs are not known. Nevertheless, the most likely explanation is that virus-specific T cells generated by HCV infection are activated after starting therapy with IFN and that these T cells could be cross-reactive with drug antigens, causing drug-induced immunopathology. These cross-reactive T cells would play a role in decreasing the viral load, while causing immunopathology in response to some drugs.

In this regard, the herpesviruses are perhaps the most likely to be involved because they induce and maintain a potent specific memory T cell response due to their common properties of ubiquitous prevalence in latent form in human populations. If this is true, antiviral T cell responses established by previous infections would shape drug-reactive repertoire and determine the severity of drug-induced immunopathology. Even after the virus is cleared, the severity of clinical symptoms of these drug-induced dermatoses could be increased by expansions of such cross-reactive T cells initially induced by past viral infections and subsequent exposure to certain drugs. Inversely, these cells would persist as a long-term, drug-specific memory T cell population by continuous stimulation with these latent viruses in patients who have no longer been exposed to the relevant drug antigens. Indeed, anti-EBV-specific CD8⁺ T cells have been shown to be cross-reactive with an unknown endogenous peptide presented by HLA-B*4402 [46]. Given that HLA-B*1502 was identified as immunogenic for carbamazepine-induced severe drug eruptions on the basis of a strong association between them in large registry analysis [47], it might be expected that memory T cells specific for a viral peptide presented in the context of certain HLA-B alleles could be involved in the pathogenesis of LDE occurring after viral infections due to their cross-reactivity to drug antigens.

Similarities to Graft-versus-Host Disease

Graft-versus-host disease (GVHD) is induced by donor immunocompetent T cells transferred into allogeneic hosts incapable of rejecting them. The target tissues are primarily the liver, gastrointestinal tract and skin. GVHD is usually divided into two forms originally defined on the basis of time of presentation: acute GVHD typically occurs between 7 and 21 days after transplantation but may be seen as late as 3 months, and chronic GVHD arises after a mean of 4 months but may occur as soon as 40 days after transplantation [18]. Chronic GVHD is further divided into two major clinical categories, lichenoid and sclerodermoid. However, the increasing use of peripheral blood rather than marrow and the early withdrawal of posttransplant immunosuppression have obscured these time-based divisions and the distinction between single categories. They may have reduced the incidence of acute GVHD, but have been associated with an elevated risk of chronic GVHD. Although alloreactive T cells that respond directly to foreign MHC molecules and bound peptide are thought to be central mediators of GVHD, autoreactive T cells that respond to self MHC molecules and bound peptide can cause syngeneic GVHD, as demonstrated in our mouse model [2–4]. Lichenoid chronic GVHD closely resembles idiopathic LP, clinically and histologically. Mucous membranes are often affected as manifested by

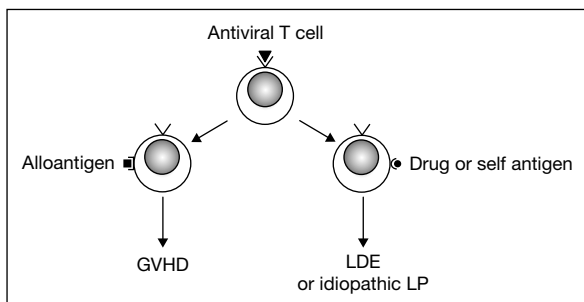


Fig. 2. The role of antiviral T cells in mediating LP and GVHD. Antiviral T cell responses established by previous infections shape their alloreactive or drug-reactive repertoire. Once antiviral T cells can cross-react with allogeneic HLA, GVHD develops. Once they can cross-react with drug antigens, LDE ensue. When they acquire reactivity with self antigens, they cause spontaneous autoimmune disease.

lacy white plaques and erosions of the oral mucosa, a finding indistinguishable from oral LP.

Interestingly, several studies demonstrated that reactivation of the herpesviruses precedes GVHD [46, 48–50], as suggested in LP. In view of close similarities between lichenoid GVHD and LP in their clinical manifestations and histopathology, these findings could be interpreted as indicating that GVHD as well as LP is triggered, or at least exacerbated, by reactivations of these herpesviruses. Thus, a cross-reaction of these antiviral T cells with either self antigens, culprit drugs or HLA molecules could play a key role in mediating LP, LDE and lichenoid GVHD, respectively (fig. 2). Support for this view is found in the observation that estimated precursor frequencies of T cells specific for EBV and CMV in healthy seropositive donors are roughly equivalent to those estimated against individual allogeneic HLA [51, 46]. Consistent with this view, we noted that long-term persistence of CD8+ T cells associated with CD68+ macrophages can be frequently seen at the healed sites of herpes simplex long after clinical resolution [Mizukawa, in preparation], a finding typically seen in oral lichenoid lesions of chronic GVHD [52]. Because Koelle et al. [53] reported that HSV-specific CD4+ and CD8+ T cells can be detected at the clonal level among cells present in the dermal infiltrate of herpetic lesions and that they are enriched about 100- to 1,000-fold in lesions compared with peripheral blood mononuclear cells, it is reasonable to speculate that such antiviral T cells retained in the lesions, when cross-reacted with other antigens such as drugs or activated nonspecifically by cytokines, would cause localized tissue injury, as observed in LP. Previous studies showed that localized trauma

and inflammation can initiate LP and suggest that localized nonspecific inflammatory responses may trigger the activation of these T cells in the vicinity.

Effector T Cells Involved in Tissue Injury

There are conflicting data regarding the T cell population in LP lesions. Although some investigators showed that the cellular infiltrate contained an increased ratio of CD4+ to CD8+ T cells [42, 54, 55], others found a predominance of CD8+ T cells, particularly in older lesions and those located in close proximity to degenerating keratinocytes [56–58]. Evidence to support the crucial role of CD8+ T cells in damage to basal keratinocytes has been provided in two ways. First, the number of intraepithelial CD8+ T cells in the region of basal membrane disruption was significantly higher than in the region of basal membrane continuity [57]. Second, CD8+ T cells isolated from lesional skin exhibited specific cytotoxic activity against autologous lesional and normal keratinocytes [58]. On the other hand, our murine model of LP has been established using autoreactive CD4+ T cells [2–4, 43], as described above. In this model, the autoreactive CD4+ T cells locally injected into the footpads of syngeneic mice can respond to self MHC class II antigens constitutively expressed on macrophages and Langerhans cells residing in the injection sites and cause epidermal damage similar to LP lesions in humans without any modification in the antigenicity of the epidermis and dermis by exogenous agents. In the natural disease process, however, modifications in the antigenicity by exogenous agents such as drugs and trauma could be a prerequisite for triggering the activation of these T cells. Because these autoreactive CD4+ T cells in our mouse model have been shown to kill epidermal keratinocytes *in vitro* and *in vivo* upon activation by producing large amounts of IFN- γ and TNF- α and by inducing or enhancing the expression of apoptosis-associated proteins such as Fas and TRAIL, they would be more efficient at causing extensive epidermal damage seen in LP. Although keratinocytes are thought to be killed via cross-linking of the Fas receptor expressed on them by its ligand (Fas L) expressed by CD8+ T cells and natural killer cells in LP lesions, apoptosis induced by Fas L and killing by the death effector molecule perforin have both been identified as cytotoxic mechanisms of EBV-specific CD4+ cytotoxic T cells [41]. Due to the constraints of the clinical study, however, it is difficult to correlate the development of tissue damage with the magnitude of the CD4+ or CD8+ T cell response in the LP lesions.

To investigate the immunopathology and kinetics of the inflammation in more detail, we should analyze a more simplified disease model, in which a complicated multistep process resulting in tissue damage can be bypassed and

the actual early events that trigger the inflammation can be examined after clinical challenge with the causative drug. Fixed drug eruption (FDE) is thought to be such a simplified disease model for LTR, because effector CD8+ T cells responsible for epidermal damage have been shown to persist as a stable population at high frequencies in the resting lesions [59–61]. This allowed us to show that the severity of epidermal injury can be exclusively induced by activation of this population, whose functions can be demonstrated in vitro, without the participation of other T cell populations. In this regard, our in situ reverse transcription PCR studies demonstrated that a strong and exclusive induction of IFN- γ mRNA and protein was seen in these intraepidermal CD8+ T cells with much faster kinetics than their dermal and peripheral counterparts after clinical challenge with the causative drug [60]. Because rapid production of large amounts of IFN- γ at an early time point (2–3 h after challenge) by these T cells preceded the epidermal invasion of other T cells such as dermal CD4+ T cells and was subsequently followed by localized epidermal injury [60], these intraepidermal CD8+ T cells are likely major effectors in epidermal injury observed in the fully evolved lesions of FDE, in which typical LTR can be seen. Because granzyme B-positive and perforin-positive cells were also identified in the same distribution pattern within the epidermis as intraepidermal CD8+ T cells in the evolving FDE lesions, severe epidermal damage observed in the fully evolved FDE lesions is likely to be caused not only by production of early burst of IFN- γ by intraepidermal CD8+ T cells, but also by perforin and granzyme B. A recent study has also shown that Fas-Fas L interactions are involved in the development of FDE lesions [62]. Our flow-cytometric analysis also demonstrated that the vast majority of intraepidermal CD8+ T cells isolated from resting FDE lesions have the capacity to produce IFN- γ upon stimulation with PMA/ionomycin, while the proportion capable of producing IL-4 was less than 1% of these T cells [60]. The phenotype of these intraepidermal CD8+ T cells, CD45RA+CD11a+CD27–CD56–, typically observed in resting FDE lesions most closely resembles that of effector memory T cells that have the capacity to migrate to nonlymphoid tissues [63–65]; nevertheless, intraepidermal CD8+ T cells are distinct from effector memory T cells in that the overwhelming majority (>80%) of the intraepidermal T cells express both cutaneous lymphocyte antigen (CLA) and $\alpha_E\beta_7$ [60]. Of note was our finding that the CD8+ T cells displayed the phenotypic conversion from CD45RA to CD45RO in vivo after clinical challenge, indicating that activation of intraepidermal CD8+ T cells is accompanied by a transient conversion to an activation-associated phenotype (that is, CD45RO). In view of the notion that effector memory T cells provide immediate frontline protection against invading pathogens at epithelial surfaces, intraepidermal CD8+ T cells abundantly detected in the resting FDE

lesions would represent skin-tropic effector memory T cells that have originally evolved to serve as self-protection mechanism(s) by eliminating stressed epidermal cells, such as virally infected cells and damaged cells. The accidental and uncontrolled activation of this population would result in severe epidermal injury often observed in FDE or LTR. Therefore, there must be regulation mechanism(s) to limit the activation of this population.

Interestingly, in the fully evolved lesions (at 24 h), the presence of CD8+ T cells was diluted by the prominent influx of CD4+ T cells to the epidermis. There was an inverse relationship between the frequencies of CD4+ T cells within the epidermis and the extent of epidermal injury: the lowest frequencies of CD4+ T cells were found in the FDE lesion, in which most severe epidermal damage was seen. Because many if not all CD4+ T cells were found to express CD25 and FoxP3 [Mizuakawa et al., in preparation], the influx into the epidermis of regulatory T cells (Tregs) during the development of FDE could represent an appropriate response that limits harmful immune reactions mediated by activation of intraepidermal CD8+ T cells. Collectively, there would be a communication between intraepidermal CD8+ T cells and other types of inflammatory cells, including Tregs, cytotoxic CD4+ T cells, macrophages and dendritic cells in the fully evolved FDE lesions. Thus, these interactions might contribute to the development of LP lesions (fig. 3), analogous to the fully evolved FDE lesions.

Mechanisms for Regulating Recruitment of T Cells

Among a variety of signals, chemokines and their receptors represent a central paradigm of the molecular basis of tissue-specific migration of memory T cells [66, 67]. Chemokines and their receptors suggested to be relevant for selective T cell migration into the skin include interactions between TARC/CCL17 expressed by cutaneous, but not intestinal, endothelium and CCR4 expressed at high levels on CLA+CD4+ skin-homing T cells [68, 69]. Nevertheless, because CCR4 is present on essentially all skin-homing T cells, but also expressed by apparently non-skin-homing T cells, it has been suggested that there may exist another chemokine receptor with better specificity for skin-homing T cells than CCR4. Indeed, another chemokine, cutaneous T cell-attracting chemokine (CTACK/CCL27) was found to be expressed by keratinocytes [70]. The receptor for CTACK/CCL27 was identified as CCR10, which is absent from non-skin-homing CD4+ T cells but only expressed by a subset of CLA+CD4+ T cells in peripheral blood [71]. These findings suggest that CCR4 and CCR10 have overlapping, redundant roles in T cell migration into the skin. Based on data using a monoclonal antibody

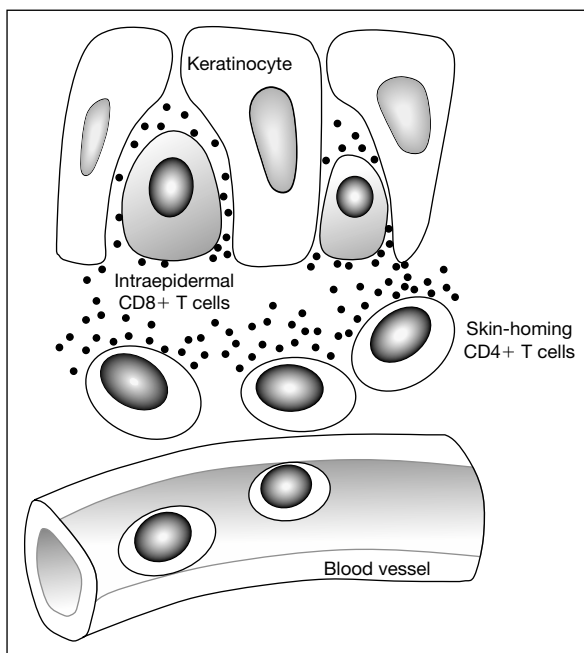


Fig. 3. Cytotoxic responses in LP are mediated by interactions between intraepidermal T cells and skin-homing CD4+ T cells. Initial epidermal damage would be induced by activation of intraepidermal CD8+ T cells originally residing in the lesions that can rapidly produce large amounts of IFN- γ and perforin (closed circles). An influx of CD4+ skin-homing T cells migrating from the circulation could cause additional damage to the epidermis by perforin and granzyme (shaded circles) or Fas L.

that recognized functional expression of CCR10 by human T cells, Soler et al. [72] suggested that CCR10 may be part of an alternative cutaneous homing pathway utilized primarily by the less numerous effector subset of the CD4+ T cell pool.

In LP lesions, a set of chemokines composed of IP-10, MCP-1, RANTES and MIG have been shown to be produced by basal keratinocytes, particularly in early lesions, and would serve to attract T cells to the dermoepidermal junction [73]. In contrast, other investigators demonstrated that infiltrating CD8+ T cells in oral LP lesions predominantly express CCR5 and CXCR3, both of which are preferentially expressed on Th1 cells, and that not only lesional keratinocytes, but also the infiltrating CD8+ T cells themselves, express the respective chemokine ligands [74]. These findings suggest a potential self-recruiting mechanism by which T cell recruitment into the lesions could be

perpetuated [74]. Thus, chemokines produced at the skin sites would regulate the composition of the Th1- or Th2-driven cell infiltrates.

A dogma has been established that emphasizes the recruitment of pathogenic T cells to the lesional skin, while ignoring the possibility that not only memory T cells with a surveillance function, but also CD4+CD25+FosP3+ Tregs, can home to the skin sites. Such immune surveillance T cells and Tregs would have unique tissue-selective address codes different from pathogenic memory T cells and the former could preferentially migrate to skin sites under noninflamed conditions, while the latter could migrate to the inflammatory skin sites in response to inducible or inflammatory chemokines. Thus, chemokines and their receptors as described above represent unlikely candidates for mediating the steady-state traffic of immune surveillance T cells under noninflamed conditions, because most inflammatory chemokines are not upregulated under these conditions. Thus, such homeostatic chemokines constitutively produced under noninflamed conditions can mediate the skin-directed migration of these immune surveillance T cells, leading to clearance of invading pathogens such as viruses. In this regard, Schaerli et al. [75] demonstrated that the majority of human T cells in healthy skin express the chemokine receptor CCR8 and that its selective ligand CCL1 is constitutively expressed on dermal microvessels and epidermal antigen-presenting cells. Based on these results, they propose that immune surveillance memory T cells are recruited and positioned within normal healthy skin by this chemokine system [75]. It should be appreciated, therefore, that we have no definitive means of distinguishing such immune surveillance T cells or Tregs from pathogenic T cells in LP lesions.

The concept of tissue-selective inhibition of inflammatory responses has already been established in mice and humans. Although earlier studies established the dogma that expression of CLA on distinct subsets of T cells contributes to their skin-homing properties by binding to E-selectin on endothelial cells, recent studies have revised this dogma and found that the CLA epitope is a posttranslational modification of the protein core of P-selectin glycoprotein-1 (PSGL-1) by the activity of $\alpha(1,3)$ -fucosyltransferase VII (Fuc T-VII), but not necessarily represents the epitope required for binding to E-selectin [76, 77]. Thus, it can be concluded that the Fuc T-VII expression is essential for generating selectin ligands absolutely required for T cell homing to skin. However, the picture is further complicated by the finding that Fuc T-IV, another Fuc T expressed to a significant degree in T cells, can also generate selectin ligands [78, 79]. A consensus has emerged from recent studies that Fuc T-VII plays a predominant role in generating E-selectin ligands (ESL), while Fuc T-IV appears to serve as an optional backup for generation of ESL [80]. These findings indicate that targeting the expression of Fuc T-VII offers an attractive therapeutic approach for controlling T cell migration to skin. In accordance with

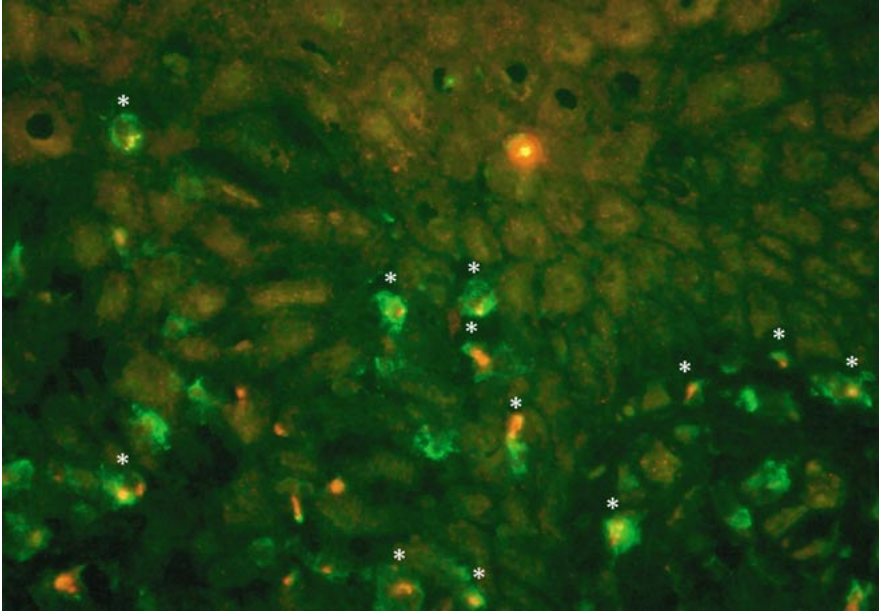


Fig. 4. Expression of Fuc T-VII protein (red) in the cytoplasm of CLA+ (green) T cells infiltrating in epidermal and dermal compartments of lesional skin in LP. The detection of Fuc T-VII and CLA expression was performed by the double immunofluorescence method. Double-positive cells are indicated by asterisks. Original magnification $\times 400$.

this view, our immunohistochemical analysis of LP lesions using specific monoclonal antibodies directed against Fuc T-VII or Fuc T-IV demonstrated that Fuc T-VII protein was expressed within the cytoplasm of CLA+ infiltrating T cells (fig. 4) in epidermal and dermal compartments of lesional skin; in contrast, Fuc T-IV protein was rarely detected in the corresponding CLA+ T cells. Compared with nonlesional skin, lesional skin exhibited a marked increase in Fuc T-VII+ skin-infiltrating T cells. Our morphologic studies performed with Fuc T-IV and Fuc T-VII transfectants showed that Fuc T-IV generates only low levels of ESL without CLA expression, while Fuc T-VII generates high levels of ESL with concomitant expression of CLA [Takahashi et al., in preparation]. In view of our observation in LP lesions, a likely interpretation of these findings is that Fuc T-VII-driven recruitment pathways could serve to induce the accumulation of relevant T cell subsets at sites of LP lesions. Nevertheless, it should also be appreciated that T cell recruitment to the skin is crucial for host defense as well and that activation of these T cells at sites of LP lesions can be viewed as preventing further dissemination of various pathogens.

Conclusions

Skin-homing T cells have long been known to be the key players in skin inflammation. Preferential expression of CLA and CCR4 on Tregs [81], however, suggests the alternative possibility that T cells with the skin-homing phenotype frequently found at sites of LP lesions could also display beneficial properties for the host. A better understanding of their physiological role in the initiation and amplification of LP is a requirement for safer and more efficient treatments.

Acknowledgements

This work was supported by grants from the Ministry of Education, Sports, Science and Culture of Japan (T.S., Y.S. and Y.K.) and the Ministry of Health, Labor, and Welfare of Japan (T.S.).

References

- 1 Marrack P, Kappler J, Kotzin BL: Autoimmune disease: why and where it occurs. *Nat Med* 2001;7:899–905.
- 2 Shiohara T, Moriya N, Gotoh C, Saizawa K, Nagashima M: Locally administered monoclonal antibodies to lymphocyte function-associated antigen 1 and to L3T4 prevent cutaneous graft-vs-host disease. *J Immunol* 1988;141:2261–2267.
- 3 Shiohara T, Moriya N, Saizawa K, Gotoh C, Yagita H, Nagashima M: Evidence for involvement of lymphocyte function-associated antigen 1 in T cell migration to epidermis. *J Immunol* 1991;146:840–845.
- 4 Shiohara T, Moriya N, Gotoh C, Hayakawa J, Nagashima M, Saizawa K, Ishikawa H: Loss of epidermal integrity by T cell-mediated attack induces long-term local resistance to subsequent attack. I. Induction of resistance correlates with increases in Thy-1⁺ epidermal cell numbers. *J Exp Med* 1990;171:1027–1041.
- 5 Selin LK, Welsh RM: Plasticity of T cell memory responses to viruses. *Immunity* 2004;20:5–16.
- 6 Welsh RM: Private specificities of heterologous immunity. *Curr Opin Immunol* 2006;18:331–337.
- 7 Pacegueiro W, Sachse MF, Amaro J, Farinha P, Fonseca I: Oral lichen planus versus oral lichenoid eruption as a manifestation of contact allergy. *Contact Dermatitis* 1999;40:333–334.
- 8 Yiannias JA, el-Azhary RA, Hand JH, Pakzad SY, Rogers RS: Relevant contact sensitivities in patients with the diagnosis of oral lichen planus. *J Am Acad Dermatol* 2000;42:177–182.
- 9 Laeigendecker R, Dekker SK, Burger PM, Mulder PGH, Van Joost T, Neumann MHA: Oral lichen planus and allergy to dental amalgam restorations. *Arch Dermatol* 2004;140:1434–1438.
- 10 Rogers RS, Bruce AJ: Lichenoid contact stomatitis: is inorganic mercury the culprit? *Arch Dermatol* 2004;140:1524–1525.
- 11 Korkij W, Chuang TY, Soltani K: Liver abnormalities in patients with lichen planus: a retrospective case-control study. *J Am Acad Dermatol* 1984;14:139–140.
- 12 Reborá A, Rongioletti F: Lichen planus and chronic active hepatitis. *J Am Acad Dermatol* 1984;10:840–841.
- 13 Criber B, Gamier C, Laustriat D, Heid E: Lichen planus and hepatitis C virus infection: an epidemiologic study. *J Am Acad Dermatol* 1994;31:1070–1072.
- 14 Pawlowsky J-M, Dhumeaux D, Bagot M: Hepatitis C virus in dermatology. *Arch Dermatol* 1995;131:1185–1193.

- 15 Nagao Y, Sata M, Noguchi S, Seno'o T, Kinoshita M, Kameyama T, Ueno T: Detection of hepatitis C virus RNA in oral lichen planus and oral cancer tissues. *J Oral Pathol Med* 2000;29:259–266.
- 16 Mignogna MD, Lo Muzio L, Lo Russo L, Fedele S, Ruoppo E, Bucci E: Oral lichen planus: different clinical features in HCV-positive and HCV-negative patients. *Int J Dermatol* 2000;39:134–139.
- 17 van der Meij EH, van der Waal I: Hepatitis C infection and oral lichen planus: a report from the Netherlands. *J Oral Pathol Med* 2000;29:255–258.
- 18 Shiohara T, Kano Y: Lichen planus and lichenoid dermatoses; in Bologna JL, Jorizzo JL, Rapini RP (eds): *Dermatology*. London, Mosby, 2003, pp 175–198.
- 19 Carrozzo M, Francia Di Celle P, Gandolfo S, Carbone M, Conrotto D, Fasano ME, Roggero S, Rendine S, Ghisetti V: Increased frequency of HLA-DR6 allele in Italian patients with hepatitis C virus-associated oral lichen planus. *Br J Dermatol* 2001;144:803–808.
- 20 Campisi G, Di Fede O, Craxi A, Di Stefano R, Margiotta V: Oral lichen planus, hepatitis C virus, and HIV: no association in a cohort study from an area of high hepatitis C virus endemicity. *J Am Acad Dermatol* 2004;51:364–370.
- 21 Pilli M, Penna A, Zerbini A, Vescovi P, Manfredi M, Negro F, Carrozzo M, Mori C, Giuberti T, Ferrari C, Missale G: Oral lichen planus pathogenesis: a role for the HCV-specific cellular immune response. *Hepatology* 2002;36:1446–1452.
- 22 Yadav M, Arivananthan M, Chandrashekan A, Tan BS, Hashim BY: Human herpesvirus-6 (HHV-6) DNA and virus-encoded antigen in oral lesions. *J Oral Pathol Med* 1997;26:393–401.
- 23 De Vries HJ, van Marle J, Teunissen MB, Picavet D, Zorgdrager F, Bos JD, Weel J, Cornelissen M: Lichen planus is associated with human herpesvirus type 7 replication and infiltration of plasmacytoid dendritic cells. *Br J Dermatol* 2006;154:361–364.
- 24 Requena L, Kutzner H, Escalonilla P, Ortiz S, Schaller J, Rohwedder A: Cutaneous reactions at sites of herpes zoster scars: an expanded spectrum. *Br J Dermatol* 1998;138:161–168.
- 25 Al-Mutairi N, Sharma AK, Osama N-E, Joshi A, Ayman H: Isotopic cutaneous lichen planus possibly related to dental amalgam. *J Am Acad Dermatol* 2004;50:653–654.
- 26 Gibney MD, Nahass GT, Leonardi CL: Cutaneous reactions following herpes zoster infections: report of three cases and a review of the literature. *Br J Dermatol* 1996;134:504–509.
- 27 Kashima HK, Kutcher M, Kessis T, Levin LS, de Villiers EM, Shah K: Human papillomavirus in squamous cell carcinoma, leukoplakia, lichen planus, and clinically normal epithelium of the oral cavity. *Ann Otol Rhinol Laryngol* 1990;99:55–61.
- 28 Boyd AS, Annarella M, Rapini RP, Adler-Storthz K, Duvic M: False-positive polymerase chain reaction results for human papillomavirus in lichen planus. *J Am Acad Dermatol* 1996;35:42–46.
- 29 Al-Khenaizan S: Lichen planus occurring after hepatitis B vaccination: a new case. *J Am Acad Dermatol* 2001;45:614–615.
- 30 Baum H, Davies H, Piekman M: Molecular mimicry in the MHC: hidden clues to autoimmunity. *Immunol Today* 1996;17:64–71.
- 31 Wucherpfennig KW: Mechanism for the induction of autoimmunity by infectious agents. *J Clin Invest* 2001;108:1097–1104.
- 32 Olsen JK, Croxford JL, Calenoff MA, Dal Carto MC, Miller SD: A virus-induced molecular mimicry model of multiple sclerosis. *J Clin Invest* 2001;108:311–318.
- 33 Panoutsakopoulou V, Sanchirico ME, Huster KM, Jansson M, Granucci F, Shim DJ, Wucherpfennig KW, Cantor H: Analysis of the relationship between viral infection and autoimmune disease. *Immunity* 2001;15:137–147.
- 34 Mason D: A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol Today* 1998;19:395–404.
- 35 Hemmer B, Jacobsen M, Sommer N: Degeneracy in T-cell antigen recognition: implications for the pathogenesis of autoimmune diseases. *J Neuroimmunol* 2000;107:148–153.
- 36 Kissler S, Anderson SM, Wraith DC: Antigen-presenting cell activation: a link between infection and autoimmunity. *J Autoimmun* 2001;16:303–308.
- 37 Pinto JM, Marques MS, Correia TE: Lichen planus and leukocytoclastic vasculitis induced by interferon α -2b in a subject with HCV-related chronic active hepatitis. *J Eur Acad Dermatol Venereol* 2003;17:193–195.
- 38 Herra Saval A, Camancho Martinez F: Lichen planus induced by interferon- α 2B therapy in a patient with cutaneous malignant melanoma. *Acta Derm Venereol* 1999;79:395.

- 39 Wenzel J, Sheler M, Proelss J, Biever T, Tüting T: Type I interferon-associated cytotoxic inflammation in lichen planus. *J Cutan Pathol* 2006;33:672–678.
- 40 Stetson DB, Medzhitov R: Antiviral defense: interferons and beyond. *J Exp Med* 2006;203:1837–1841.
- 41 Heller KN, Gurer C, Münz C: Virus-specific CD4⁺ T cells: ready for direct attack. *J Exp Med* 2006;203:805–808.
- 42 Massari LP, Kastelan M, Gruber F, Laskarin G, Tokmadzic VS, Strbo N, Zamdo G, Zavhar G: Perforin expression in peripheral blood lymphocytes and skin-infiltrating cells in patients with lichen planus. *Br J Dermatol* 2004;151:433–439.
- 43 Shiohara T, Moriya N: Epidermal T cells: their functional role and disease relevance for dermatologists. *J Invest Dermatol* 1997;109:271–275.
- 44 Scotet E, Peyrat MA, Saulquin X, Retiere C, Couedel C, Davodeau F, Dulphy N, Toubert A, Bignon JD, Lim A, Vie H, Hallet MM, Liblau R, Weber M, Berthelot JM, Houssaint E, Bonneville M: Frequent enrichment for CD8 T cells reactive against common herpes viruses in chronic inflammatory lesions: towards a reassessment of the physiopathological significance of T cell clonal expansions found in autoimmune inflammatory processes. *Eur J Immunol* 1999;29:973–985.
- 45 Polakos NK, Cornejo JC, Murray DA, Wright KO, Treanor JJ, Crispe IN, Topham DJ, Pierce RH: Kupffer cell-dependent hepatitis occurs during influenza infection. *Am J Pathol* 2006;168:1169–1178.
- 46 Burrows SR, Silins SL, Moss DJ, Khanna R, Misko IS, Argant VP: T cell receptor repertoire for a viral epitope in humans is diversified by tolerance to a background major histocompatibility complex antigens. *J Exp Med* 1995;182:1703–1705.
- 47 Chung WH, Hung SI, Hong HS, Hsieh MS, Yang LC, Ho HC, Wu JY, Chen YT: Medical genetics: a marker for Stevens-Johnson syndrome. *Nature* 2004;428:486.
- 48 Maeda Y, Teshima T, Yamada M, Harada M: Reactivation of human herpesviruses after allogeneic peripheral blood stem cell transplantation and bone marrow transplantation. *Leuk Lymphoma* 2000;29:229–239.
- 49 Yoshikawa T, Suga S, Asano Y, Nakashima Y, Yazaki T, Sobue R, Hirono M, Fududa M, Kojima S, Matsuyama T: Human herpesvirus-6 infection in bone marrow transplantation. *Blood* 1991;78:1381–1384.
- 50 Singh N, Carrigan DR: Human herpesvirus-6 in transplantation: an emerging pathogen. *Ann Int Med* 1996;124:1065–1071.
- 51 Sharrock CE, Kaminski E, Man S: Limiting dilution analysis of human T cells: a useful clinical tool. *Immunol Today* 1990;11:281–286.
- 52 Sato M, Tokuda N, Fukumoto T, Mano T, Sato T, Ueyama Y: Immunohistopathological study of the oral lichenoid lesions of chronic GVHD. *J Oral Pathol Med* 2006;35:33–36.
- 53 Koelle DM, Posavad CM, Barnum GR, Johnson ML, Frank JM, Corey JM: Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocytes. *J Clin Invest* 1998;101:1500–1508.
- 54 Shiohara T, Moriya N, Tanaka Y, Arai Y, Hayakawa J, Chiba M, Nagashima M: Immunopathologic study of lichenoid skin diseases: correlation between HLA-DR-positive keratinocytes or Langerhans cells and epidermotropic T cells. *J Am Acad Dermatol* 1988;18:67–74.
- 55 Shiohara T, Moriya N, Nagashima M: Induction and control of lichenoid tissue reactions. *Springer Semin Immunopathol* 1992;13:369–385.
- 56 Bovenschen HJ, Seyger MMB, von Kerkhof PCM: Plaque psoriasis vs. atopic dermatitis and lichen planus: a comparison for lesional T-cell subsets, epidermal proliferation and differentiation. *Br J Dermatol* 2005;153:72–78.
- 57 Zhou XJ, Sugerman PB, Savage NW, Walsh LJ, Seymour GJ: Intra-epithelial CD8⁺ T cells and basement membrane disruption in oral lichen planus. *J Oral Pathol Med* 2002;31:23–27.
- 58 Sugerman PB, Satterwhite K, Bigby M: Autocytotoxic T-cell clones in lichen planus. *Br J Dermatol* 2000;142:449–456.
- 59 Teraki Y, Moriya N, Shiohara T: Drug-induced expression of intercellular adhesion molecule-1 on lesional keratinocytes in fixed drug eruption. *Am J Pathol* 1994;145:550–560.
- 60 Mizukawa Y, Yamazaki Y, Teraki Y, Hayakawa J, Hayakawa K, Nuriya H, Kohara M, Shiohara T: Direct evidence for interferon-gamma production by effector-memory-type intraepidermal T cells

residing at an effector site of immunopathology in fixed drug eruption. *Am J Pathol* 2002;161:1337–1347.

- 61 Shiohara T, Mizukawa Y, Teraki Y: Pathophysiology of fixed drug eruption: the role of skin-resident T cells. *Curr Opin Allergy Clin Immunol* 2002;2:317–323.
- 62 Choi HJ, Ku JK, Kim MY, Kang H, Cho SH, Kim HO, Park YM: Possible role of Fas/Fas ligand-mediated apoptosis in the pathogenesis of fixed drug eruption. *Br J Dermatol* 2006;154:419–425.
- 63 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;401:708–712.
- 64 Mackay CR, von Adrian UH: Memory T cells – local heroes in the struggle for immunity. *Science* 2001;291:2323–2324.
- 65 Masopust D, Vezys V, Marzo AL, Leofrançois L: Preferential localization of effector memory T cells in nonlymphoid tissue. *Science* 2001;291:2413–2417.
- 66 Cambell JJ, Butcher EC: Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. *Curr Opin Immunol* 2000;12:336–341.
- 67 Kunkel EJ, Butcher EC: Chemokines and the tissue-specific migration of lymphocytes. *Immunity* 2002;16:1–4.
- 68 Campbell JJ, Haraldsen G, Pan J, Rottman J, Qin S, Ponath P, Andrew DP, Warnke R, Ruffing N, Kassam N, Wu L, Butcher EC: The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 1999;400:776–780.
- 69 Campbell JJ, Brightling CE, Symon FA, Qin S, Murphy KE, Hodge M, Andrew DP, Wu L, Butcher EC, Wardlaw AJ: Expression of chemokine receptors by lung T cells from normal and asthmatic subjects. *J Immunol* 2001;166:2842–2848.
- 70 Homey B, Wang W, Soto H, Buchanan ME, Wiesenborn A, Catron D, Muller A, McClanahan TK, Dieu-Nosjean MC, Orozco R, Ruzicka T, Lehmann P, Oldham E, Zlotnik A: Cutting edge: the orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin-associated chemokine CCL27 (CTACK/ALP/ILC). *J Immunol* 2000;164:3465–3470.
- 71 Homey B, Alenius H, Muller A, Soto H, Bowman EP, Yuan W, McEvoy L, Lauerma AI, Assmann T, Bunemann E, Lehto M, Wolff H, Yen D, Marxhausen H, To W, Sedgwick J, Ruzicka T, Lehmann P, Zlotnik A: CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. *Nat Med* 2002;8:157–165.
- 72 Soler D, Humphreys TL, Spinola SM, Campbell JJ: CCR4 versus CCR10 in human cutaneous TH lymphocyte trafficking. *Blood* 2003;101:1677–1682.
- 73 Spandau U, Toksoy A, Goebeler M, Brocker EB, Gillitzer R: MIG is a dominant lymphocyte-attractant chemokine in lichen planus lesions. *J Invest Dermatol* 1998;111:1003–1009.
- 74 Iijima W, Ohtani H, Nakayama T, Sugawara Y, Sato E, Nagura H, Yoshie O, Sasano T: Infiltrating CD8⁺ T cells in oral lichen planus predominantly express CCR5 and CXCR3 and carry respective chemokine ligands RANTES/CCL5 and IP-10/CXCL10 in their cytolytic granules. *Am J Pathol* 2003;163:261–268.
- 75 Schaeferli P, Ebert L, Willmann K, Blaser A, Roos RS, Loetscher P, Moser B: A skin-selective homing mechanisms for human immune surveillance T cells. *J Exp Med* 2004;199:1265–1275.
- 76 Wagers AJ, Waters CM, Stoolman LM, Kansas GS: Interleukin 12 and interleukin 4 control T cell adhesion to endothelial selectins through opposite effects on α 1,3-fucosyltransferase VII gene expression. *J Exp Med* 1998;188:2225–2231.
- 77 Takahashi R, Mizukawa Y, Yamazaki Y, Hayakawa K, Hayakawa J, Kudo A, Shiohara T: In vitro differentiation from naïve to mature E-selectin binding CD4 T cells: acquisition of skin-homing properties occurs independently of cutaneous lymphocyte antigen expression. *J Immunol* 2003;171:5769–5777.
- 78 Smithson G, Rogers CE, Smith PL, Scheidegger EP, Petryniak B, Myers JT, Kim DS, Homeister JW, Lowe JB: Fuc-TVII is required for T helper 1 and T cytotoxic 1 lymphocyte selectin ligand expression and recruitment in inflammation, and together with Fuc-TIV regulates naïve T cell trafficking to lymph nodes. *J Exp Med* 2001;194:601–614.
- 79 Homeister JW, Thall AD, Petryniak B, Maly P, Rogers CE, Smith PL, Kelly RJ, Gersten KM, Askari SW, Cheng G, Smithson G, Marks RM, Misra AK, Hindsquall O, von Andrian UH, Lowe JB: The α 1,3-fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. *Immunity* 2001;15:115–126.

- 80 Martinez M, Joffraud M, Giraud S, Baisse B, Bernimoulin MP, Schapira M, Spertini O: Regulation of PSGL-1 interactions with L-selectin, P-selectin, and E-selectin: role of human fucosyltransferase-IV and -VII. *J Biol Chem* 2005;280:5378–5390.
- 81 Hirahara K, Liu L, Clark RA, Yamanaka K, Fruhlbrigge RC, Kupper TS: The majority of human peripheral blood CD4⁺CD25^{high}Foxp3⁺ regulatory T cells bear functional skin-homing receptors. *J Immunol* 2006;177:4488–4494.

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Autoimmune Etiology of Generalized Vitiligo

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Abstract

Vitiligo is characterized by progressive skin depigmentation resulting from an autoimmune response targeting epidermal melanocytes. Melanocytes are particularly immunogenic by virtue of the contents of their melanosomes, generating the complex radical scavenging molecule melanin in a process that involves melanogenic enzymes and structural components, including tyrosinase, MART-1, gp100, TRP-2 and TRP-1. These molecules are also prime targets of the immune response in both vitiligo and melanoma. The immunogenicity of melanosomal proteins can partly be explained by the dual role of melanosomes, involved both in melanin synthesis and processing of exogenous antigens. Melanocytes are capable of presenting antigens in the context of MHC class II, providing HLA-DR+ melanocytes in perilesional vitiligo skin the option of presenting melanosomal antigens in response to trauma and local inflammation. Type I cytokine-mediated immunity to melanocytes in vitiligo involves T cells reactive with melanosomal antigens, similar to T cells observed in melanoma. In vitiligo, however, T cell tuning allows T cells with higher affinity for melanocyte differentiation antigens to enter the circulation after escaping clonal deletion in primary lymphoid organs. The resulting efficacious and progressive autoimmune response to melanocytes provides a roadmap for melanoma therapy.

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Introducing Autoimmune Vitiligo

Progressive depigmentation of the skin is considered the hallmark of vitiligo, an autoimmune disease that strikes approximately 1% of the global population. An increased incidence of vitiligo is noted among select consanguineous communities [1]. Responses to a vitiligo questionnaire support a female gender bias of approximately 1.25 [unpubl. data]. This is in concordance with an increased general

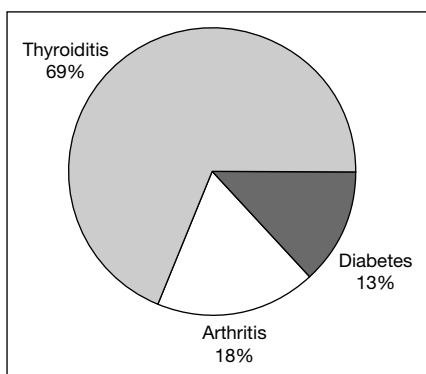


Fig. 1. Autoimmune disease in vitiligo patients. Disease prevalence among approximately one third of vitiligo patients who have reported suffering from other autoimmune diseases. Clearly, autoimmune thyroiditis is the most prevalent expression of other autoimmune disease among vitiligo patients. Diseases other than those depicted include low frequencies of psoriasis and Raynaud's disease (data not shown).

prevalence of autoimmune disease noted among women, a finding which has not been adequately explained to date. The mean age of onset for human vitiligo is 28, while the median age is 13 years, reflecting a proportion of patients that develop late-onset vitiligo [2, 3]. Early-onset vitiligo appears more clearly associated with hereditary factors, whereas environmental factors contribute particularly to late-onset vitiligo which displays a different distribution of the lesions [4]. Vitiligo is generally classified according to the extent, type and distribution of the lesions, ranging from focal vitiligo to segmental, inflammatory and generalized vitiligo, and finally to universal vitiligo. Segmental vitiligo is exceptional because of its asymmetric distribution and characteristically slow progression, suggestive of a separate, converging etiology [5].

Several factors may contribute to the pathogenesis of autoimmune vitiligo, including genetic predisposition, toxic metabolites interfering with melanin metabolism, neurochemical factors and specific autoimmunity against melanocytes [6]. Interestingly, patients generally report itch immediately preceding depigmentation of the skin, suggesting a release of histamine and other inflammatory mediators by mast cells in active disease [unpubl. observation]. Furthermore, repigmentation therapies, such as steroids and UV irradiation, are immunosuppressive and the beneficial effect of such treatment indicates the involvement of autoimmunity in vitiligo [7]. Such an involvement of autoimmune reactivity in melanocyte destruction leading to vitiligo is further supported by an association between vitiligo and other autoimmune diseases (fig. 1), most notably Hashimoto's thyroiditis [8]. Among vitiligo patients, the incidence of Hashimoto's thyroiditis is increased 2.5-fold compared to the general population. Other diseases reported to associate with vitiligo include diabetes, psoriasis and Raynaud's disease.

Autoimmune reactivity in vitiligo was initially demonstrated by an increased prevalence of circulating autoantibodies to melanocytes in association with

progressive disease [9]. Target antigens reported for the humoral response are primarily of intracellular, melanosomal origin. Antibodies have limited access to target antigens expressed within viable cells, and antibodies to intracellular antigens are likely generated in response to melanocyte damage. An important exception is the membrane receptor for melanin concentrating hormone (MCHR) [10]. Antibodies binding to membrane antigens expressed exclusively by melanocytes can contribute to melanocyte destruction by antibody-mediated cellular cytotoxicity. In this regard, reduced expression of the complement-associated factors decay-accelerating factor (DAF/CD55), CD59 and membrane cofactor protein (MCP/CD46) has been demonstrated in vitiligo skin, suggesting that vitiligo melanocytes are increasingly sensitive to complement-mediated cytotoxicity [11].

A contribution of cytotoxic T cells in melanocyte destruction was long overlooked. As melanocytes are dispersed throughout the basal layer of the epidermis, few T cells are required to target only the perilesional area surrounding actively depigmenting lesions. Immune infiltrates contain CD8+ T cells, macrophages and to a lesser extent CD4+ T cells. This was first described for patients with overt inflammatory borders surrounding the lesions [12]. Colocalization of disappearing melanocytes was found with CD8+ T cells that expressed the skin-homing marker, cutaneous leukocyte-associated antigen (CLA), and the T cell activation markers perforin and granzyme B [13]. Infiltrating T cells were also shown to express the IL-2 receptor α -chain, CD25. Now commonly associated with regulatory T cells, expression of CD25 is more likely indicative of T cell activation in progressing vitiligo, where regulatory T cells are infrequently found in the skin [unpubl. observation]. T cell infiltrates per se are commonly observed in progressive generalized vitiligo, constituting the majority of patients. Vitiligo patients carry increased numbers of peripheral T cells reactive with melanocyte differentiation antigens, tyrosinase, gp100 or MART-1 (Melanoma Antigen Recognized by T cells), compared to healthy donors [14–17]. The finding of CLA expression by circulating MART-1-specific T cells in vitiligo patients supports the role of skin-homing autoreactive T cells in the pathogenesis of vitiligo [16]. Melanocyte-specific cytotoxic T cells are also found in the perilesional vitiligo skin during active disease, suggesting their involvement in the depigmentation process.

As vitiligo is not restricted to humans, but also reported in horses, dogs and chickens, animal models are available to study the etiopathology of the disease in more detail. In particular the Smyth line chicken has proven useful to establish the involvement of an autoimmune response to melanocytes. Several observations made in the Smyth Line chicken are in concordance with human disease, including expression of a type I cytokine pattern, cytotoxic T cell involvement and a contribution of stress as a precipitating factor [18].

Vitiligo and Melanoma

The contribution of effector T cells to progressive vitiligo provides an interesting link to effective antitumor immunity in melanoma, where the majority of tumor-infiltrating T cells were found to respond to the melanosomal differentiation antigens gp100 or MART-1 [19]. It has long been recognized that vitiligo can develop in melanoma patients with an active immune response to their tumor and visible development of autoimmunity is considered a positive prognostic factor among melanoma patients [20–22]. This was recently substantiated by a markedly increased survival rate observed among melanoma patients that developed autoimmunity in response to IFN- α treatment [23].

Clonally expanded T cells as well as autoantibodies reactive with melanocyte differentiation antigens expressed by melanocytes and melanoma cells were found in the circulation of melanoma patients with leukoderma, as well as in autoimmune vitiligo skin [24–27]. The same holds true for circulating autoantibodies [28]. The development of vitiligo has frequently been observed in response to melanoma immunotherapy by vaccination with melanoma antigens, or by adoptive transfer of melanoma-specific T cells [27, 29–33], suggesting that the antimelanoma immune response can also attack normal melanocytes. In the adoptive transfer studies, infused T cells were found to infiltrate the depigmented skin lesions, indicating that activated T cells can cause human vitiligo. Interestingly, vitiligo was also observed after lymphocyte infusion for relapsed leukemia [34]. The development of vitiligo following vaccination in combination with anti-CTLA-4 blockade or by lymphodepletion prior to adoptive transfer suggests that a decreased regulatory T cell function favors the induction of vitiligo [29, 31]. Taken together, these studies show that immune responses to common antigens present on melanoma cells and normal melanocytes following specific immunotherapy may lead to skin depigmentation and tumor rejection.

The frequency of MART-1-reactive T cells among melanoma patients is not significantly different from vitiligo patients. Similar numbers of anti-MART-1 T cells from vitiligo patients, however, effectuate a higher avidity towards peptide-loaded target cells than T cells from melanoma patients, and a distinctly higher cytotoxicity of vitiligo T cells towards melanoma cells has been observed [35]. This difference in the avidity of the T cell response in vitiligo compared to melanoma may result from T cell tuning and activation mechanisms in vitiligo patients, as described below [36, 37]. This is of particular interest as we study the T cell receptor (TCR) genes expressed by vitiligo and melanoma lesional T cells. Similarity among particularly α -chains of TCR reactive with MART-1 among both T cell populations has been reported. As suggested by Palermo et al. [35], affinity maturation characterized by restricted

TCR repertoires and increased avidity may be initiated in either disease, but is not likely to progress in the immunosuppressive melanoma environment.

In animals that develop spontaneously regressing melanomas, such as Lippizaner horses or the Sinclair swine, tumor regression is similarly associated with marked depigmentation [38]. Moreover, new antimelanoma vaccines tested in mice similarly support a link between immune reactivity to normal and transformed melanocytes. In fact, vaccine-induced depigmentation serves as a model for autoimmune vitiligo. For example, after adoptive transfer of high-avidity T cells against tyrosinase derived from albino mice, recipient pigmented mice develop depigmentation with a distribution pattern strikingly similar to vitiligo [39]. Vaccination of gp100-specific TCR transgenic mice bearing large subcutaneous B16 melanoma tumors with gp100 peptide and IL-2 induced T cell activation *in vivo*, leading to tumor regression as well as vitiligo [40]. Moreover, another murine model of vaccination with GM-CSF-producing B16 melanoma cells demonstrated that the efficacy of antitumor therapy correlated with the frequency of tyrosinase-related protein-specific cytotoxic T cells in the periphery, as well as with the extent of autoimmune skin depigmentation [41]. The beneficial effect of a decreased regulatory T cell function either by anti-CTLA-4 blockade or by lymphodepletion prior to adoptive transfer on the development of vitiligo and melanoma regression was also demonstrated in these murine models. Interestingly, progressive depigmentation can be observed in mice treated with antimelanoma vaccines in the absence of tumors, as first demonstrated by Overwijk et al. [42]. This principle is illustrated in figure 2.

In the pathogenesis of vitiligo, T cells are adequately activated to exert effector function *in vivo*. Therefore, the activated T cell response in vitiligo represents a clinically relevant example of an effector T cell population with proven *in vivo* efficacy, that can serve as an example for antimelanoma T cell responses.

Challenges to the Skin: The Elicitation Phase

Approximately 50% of vitiligo patients experience a Koebner phenomenon, exhibiting new and expanding lesions initiated from a site of previous trauma, excessive sun exposure or contact with bleaching phenols [unpubl. observation]. These data support the concept that stress to the skin can precipitate the disease.

Trauma to the skin can locally generate oxygen radicals, and a reduced ability of vitiligo melanocytes to detoxify highly reactive radicals has been proposed, supported by observations of reduced dihydrobiopterin synthesis, increased H₂O₂ stress and reduced catalase activity in vitiligo skin [43]. These

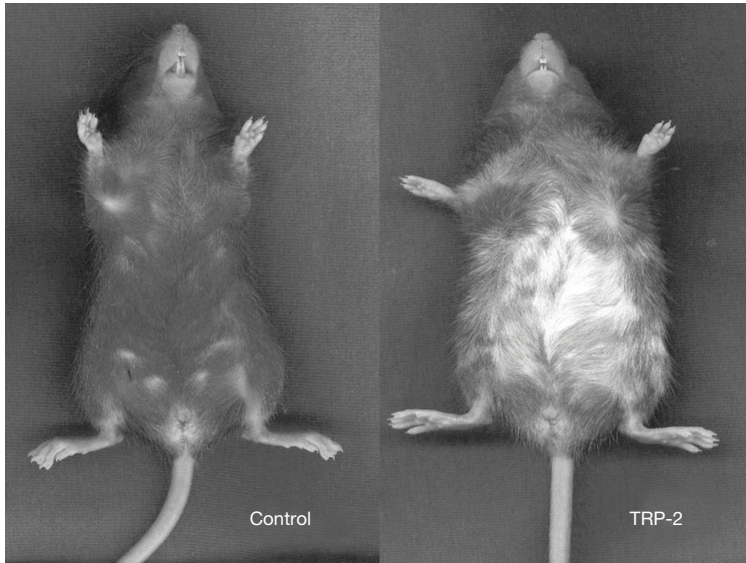


Fig. 2. Mice vaccinated against melanocyte differentiation antigens exhibit vitiligo-like depigmentation. Mice were gene gun vaccinated with empty bullets (control) or bullets coated with an expression plasmid encoding human TRP-2 once a week for 4 consecutive weeks. Lasting depigmentation is apparent only in the TRP-2-vaccinated mice, gradually spreading from the vaccination area to other areas of the mouse pelage.

findings have ultimately led to the development of pseudocatalase treatment for vitiligo to restore the redox balance. Interestingly, protein disulfide isomerase (PDI) is an integral part of the major histocompatibility complex (MHC) class I loading complex, and the activity of this enzyme affects the oxidation status of the disulfide bond in the groove of the HLA-A*0201 molecule, thereby determining its accessibility to peptides [44]. It can thus be hypothesized that an altered redox potential following stress influences the efficiency of peptides binding the groove of MHC class I and thereby affects the visibility of the cell to infiltrating effector T cells.

Among patients not exhibiting the Koebner phenomenon, it is well possible that an emotionally traumatic event such as a recent death in the family can trigger the onset of disease. Emotional stress (anxiety) and physical stress (trauma) are generally associated with elevated plasma levels of proopiomelanocortin (POMC) the precursor molecule to melanocortin peptides, including ACTH, α - and β -melanocyte-stimulating hormones (MSH), and β -endorphin. In the skin, keratinocytes and melanocytes can also produce these melanocortins in response to UV radiation, which subsequently enhance the biosynthesis

of eumelanin in melanocytes [45]. α -MSH is not only known to stimulate melanogenesis and melanocyte proliferation, it can also suppress immune responses through its effects on melanocortin receptor-expressing cells. In this respect α -MSH induces downregulation of cytokine production and the expression of costimulatory molecules on dendritic cells. Surprisingly, vitiligo patients display reduced plasma levels of α -MSH, which suggests decreased POMC processing in vitiligo skin [46]. Vitiligo patients also accumulate H_2O_2 in the skin, which oxidizes α -MSH and further decreases its availability [47]. Conversely, α -MSH can protect melanocytes from oxidative damage by its antioxidant effect during melanin synthesis, and therefore the reduced availability of α -MSH in vitiligo skin may enhance oxidative stress in vitiligo skin. Reduced levels of α -MSH in vitiligo may therefore facilitate a local immune response against melanocytes [48].

The situation of oxidative stress puts intracellular protein synthesis on hold in favor of heat shock protein (HSP) synthesis, offering epidermal cells protection from impending apoptosis. Among protein expression induced by stress, HSP70 is actively secreted by melanocytes and stimulates dendritic cells to cross-present chaperoned antigens, thereby initiating an immune response against melanocytes [49]. This is of particular relevance for forms of stress with a selective effect on melanocytes, such as exposure to bleaching phenols with a structural similarity to substrates of melanogenesis. In this respect, 4-tertiary butyl phenol (4-TBP), a causative agent in occupational vitiligo, appears to be metabolized by enzymes of the melanogenic pathway converting the compounds into cytotoxic compounds exclusively in melanocytes [50]. At lower concentrations, 4-TBP suppresses melanogenesis through competitive inhibition of tyrosinase, whereas at higher concentrations the compound is selectively cytotoxic towards melanocytes [50]. Similarly, monobenzyl ether of hydroquinone induces depigmentation upon topical application [51]. This depigmentation treatment has been used for vitiligo patients with extensive vitiligo. Phenolic agents, UV and to some extent mechanical injury all increase local levels of reactive oxygen species. Among these reactive oxygen species, nitric oxide may enhance depigmentation by reducing the adhesive capacity of melanocytes [52]. Nitric oxide is toxic to melanocytes and nitric oxide synthase is inhibited by MSH, suggesting that abundant nitric oxide contributes to vitiligo pathogenesis [53]. Other eliciting factors in vitiligo include hormones, such as estrogens, and neural factors, such as catecholamines, of which increased levels were associated with active vitiligo. The contribution of this and other mechanisms may be elucidated by studying the mode of cell death in vitiligo melanocytes.

The main mechanism of melanocyte death is likely apoptosis, although the sparsity of dying melanocytes in any given stretch of skin complicates capture

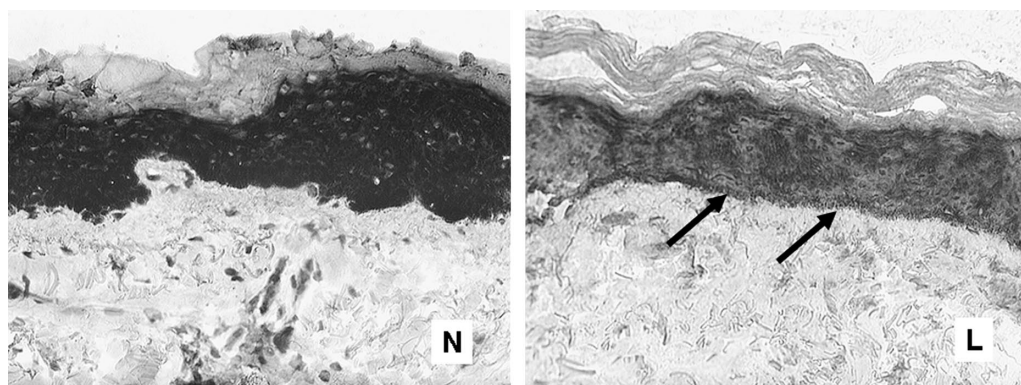


Fig. 3. Expression of HSP70 in progressive vitiligo skin. Expression of HSP70 in non-lesional (N) versus lesional (L) skin from the same patient. Expression in lesional skin was patchy and diminished compared to expression in nonlesional skin. The pattern observed in nonlesional skin was also observed in skin from control individuals, demonstrating very intense and homogeneous staining (data not shown).

of apoptotic melanocytes in the skin in situ [54]. This hypothesis is supported by the fact that melanocyte-specific T cells are frequently found in perilesional vitiligo skin and that T cells are known to kill target cells by apoptosis through the release of granzyme B. Moreover, increased expression of integrins and decreased apoptosis were shown to correlate with melanocyte retention in cultured skin substitutes [55].

Immune Activation

In response to 4-TBP exposure, we have found that melanocytes upregulate expression of HSP70 and a larger proportion of the HSP70 appears to be secreted by vitiligo melanocytes than by control melanocytes [56]. Since HSP70 is known to enhance immunity to associating proteins, as shown in melanoma vaccination studies of HSP70 bound to melanosomal proteins, it may also be involved in accelerating depigmentation in vivo [49]. In response to HSP70, dendritic cells upregulate expression of the TNF family member TRAIL, enabling dendritic cells to kill melanocytes with elevated expression of TRAILR1 and TRAILR2 in response to 4-TBP. An indication of the in vivo relevance of these findings is the altered, patchy expression of HSP70 in lesional epidermis (in 3 of 3 patients) compared to nonlesional skin (fig. 3).

HSP70 is known to induce dendritic cell activation by binding to HSP receptors on these cells [49]. CD91, a receptor for HSP70, was abundantly expressed

among vitiligo-infiltrating dendritic cells (data not shown), indicating that HSP-mediated dendritic cell activation through receptors for HSP70 and other relevant immunogenic stress proteins may be involved in the pathogenesis of vitiligo.

In the presence of HSP70, an increased number of melanocyte-reactive T cells is recruited from skin-draining lymph nodes and upon arrival in the skin, the cytotoxic effector function of these T cells is enhanced. Moreover, extracellular HSP70 can induce the expression of the costimulatory molecule ICAM-1 specifically in melanocytes among a culture of ICAM-1-negative keratinocytes [unpubl. observation]. In line with the proposed involvement of HSP70 in vitiligo, ICAM-1 is also consistently upregulated in perilesional vitiligo skin [13]. As a costimulatory pathway, ICAM-1/LFA-1 (intercellular adhesion molecule-1/leukocyte function associated antigen-1) interactions determine in part the interaction between a T cell and its target, and thus the avidity of the T cell response. In this regard we have proposed that differential ICAM-1 expression by melanocytes in vitiligo can contribute to T cell-mediated depigmentation [57]. Costimulation is also determined by interaction of CD28 with either B7 molecules on the melanocyte cell surface or its alternative, immunomodulating ligand CTLA-4. Genetically determined CTLA-4 deficiencies in autoimmune disease (including vitiligo) may result in preferential binding of CD28 to B7, supporting effective costimulation rather than CTLA-4-mediated immunoregulation [58].

The cytokine microenvironment in perilesional vitiligo skin is also conducive for dendritic cell activation and T cell-mediated cytotoxicity. Macrophages and dendritic cells are abundantly present in depigmenting vitiligo skin. The activation state of macrophages and dendritic cells within vitiligo skin has not been described to date. Since Langerhans cells, dendritic cells and melanophages may each be involved in phagocytosis, antigen processing and/or presentation of melanocyte-derived antigens in skin-draining lymph nodes, it is important to assess the physiology of these cell types within perilesional areas of actively depigmenting skin. In this respect it has been shown that Langerhans cells in perilesional vitiligo skin express elevated levels of β -1,6-branched N-glycans, which appears to be indicative of Langerhans cell activation in progressive disease [Pawelek, pers. commun.]. Similar glycosylation patterns expressed by macrophages are considered a negative prognostic indicator in breast cancer and melanoma [59]. The apparent discrepancy between β -1,6-branched N-glycan expression in active vitiligo representing immune activation and its expression in tumors generally representing a lack of effective immunity is best understood realizing that the glycosylation pattern reflects the abundance of cells engaged in phagocytosis, regardless of cell type, whereas tumor-infiltrating macrophages and vitiligo-derived Langerhans cells differ in their ability to activate a specific immune response [60]. Considering that Langerhans cells are located in close proximity to melanocytes in the epidermis, that in vitiligo lesional skin Langerhans

cells are predominantly located in the basal layer of the epidermis, and that reduced contact sensitization was noted for lesional vitiligo skin, it is likely that Langerhans cells are involved in vitiligo etiology by processing and cross-presentation of melanocyte antigens [61].

A contribution of immune activation to progressive depigmentation is also supported by expression of MHC class II in perilesional epidermis. Perilesional expression of HLA-DR is relevant, as melanocytes can present antigens in the context of MHC class II. Besides presenting endogenous melanosomal peptides in the context of MHC class II, melanocytes are also capable of processing exogenous antigens and presenting them in the context of MHC class II. This is likely relevant to explain the extraordinary link to depigmentation observed in tuberculoid leprosy if melanocytes are killed after processing and presenting immunodominant antigens of *Mycobacterium leprae*, including hsp65. In support of this theory, we have demonstrated class II restricted killing of hsp65 presenting melanocytes in vitro [57].

Several data support an abundance of type 1 cytokines in perilesional skin, indicating a cell-mediated immune response to melanocytes [62]. The polarization of the T cell response towards a type 1 response during progression of depigmentation in vitiligo has been demonstrated in a study of vitiligo skin-infiltrating T cells that were isolated and cultured from vitiligo skin [63]. In this study, T cell clones derived from vitiligo-infiltrating T cells skin predominantly exhibited type 1-like cytokine secretion profiles. Interestingly, already in uninvolved skin of these vitiligo patients microdepigmentation was observed in situ, correlating with the extent of type 1 polarization of local skin-derived T cells. The ratio of CD4+ to CD8+ T cells is decreased in peripheral blood of vitiligo patients regardless of disease status [62], signifying a relative increase in cytotoxic T cells. Immunohistochemical analysis of skin tissue sections similarly revealed a decreased CD4+ to CD8+ T cell ratio, indicating that the shift in the balance between CD4+ and CD8+ T cells cultured from vitiligo skin was not due to differences in the in vitro expansion capacity of these T cell subsets. Based on these findings, the cytokine profiles observed among cultured cells are considered representative of those found in progressive vitiligo skin. Moreover, the type 1 cytokine secretion patterns of T cells in vitiligo were confirmed in the prime animal model for vitiligo, the Smyth line chicken [18].

Cytotoxic T cells infiltrating the skin in patients with progressive vitiligo are found in close proximity to remaining melanocytes in the skin [11]. The CD8+ T cells derived from progressively depigmenting skin were cytotoxic towards autologous melanocytes in vitro. We have recently found that melanocyte-specific T cells can induce apoptosis of melanocytes in situ in non-lesional skin of vitiligo patients [unpubl. data], indicating that the effector phase of melanocyte destruction is mediated by cytotoxic T cells.

It should be noted that T cells reactive with melanocyte differentiation antigens can be found in the peripheral blood of healthy donors and their presence per se can therefore not be considered evidence of autoimmune phenomena. For the MART-1 antigen, it was shown that a large pool of MART-1-specific T cells are generated in the thymus, which circulate as naïve T cells in the peripheral blood of healthy individuals [64]. Tumor-antigen-driven activation and expansion of this T cell pool is seen in melanoma patients and may also occur during vitiligo development, where activated MART-1-specific T cells are found in the skin.

In autoimmune disease, T cells expressing high-affinity TCR fail to be clonally deleted in primary lymphoid organs and as a result, high-avidity T cells recognizing self antigens enter the circulation T cells. This is observed in autoimmune polyendocrinopathy, where the development of vitiligo highlights the severity of disease [65]. Negative selection is dependent at least in part on presentation of peripheral antigens in the thymus, allowing only T cells with low avidity towards encountered antigens to enter the periphery [66]. In this respect, autoimmune regulator-dependent presentation of the melanocyte differentiation antigen gp100 has been demonstrated. It is possible that a particular autoimmune disease reflects a lack of presentation of a select antigen or set of antigens in the thymus, however, additional mechanisms are likely to contribute to organ-specific autoimmunity. For example, selection against high-affinity TCR can be masked by T cell tuning [36]. Here high-avidity T cells with high-affinity TCR pass clonal selection criteria by downplaying their avidity in the thymus, for example by expression of CD5 to reduce TCR signaling, or by altering CD4 or CD8 expression levels during selection. Once tuned, T cells migrate to the site expressing their native antigens and these cells propagate and undergo limited affinity maturation to express higher-affinity TCR. Although T cell tuning may explain how high-avidity T cells enter the circulation, it does not automatically explain autoimmunity. When tuned high-avidity T cells enter the circulation, the hyporesponsive state must be overcome for autoimmunity to arise. This can occur when the target antigen is overexpressed as in melanoma. Alternatively, the danger model proposed by Matzinger [67] suggests that any given antigen, self or nonself, evokes an immune response when it is presented to the immune system in combination with a danger signal. This is reflected primarily in the state of activation of antigen-presenting cells, and dendritic cell activation signals thus constitute a turning point in the immune response. Mechanical injury, UV exposure and contact with bleaching phenols may thus precipitate vitiligo by inducing such danger signals leading to activation of dendritic cells. Depending on the activation signal, dendritic cells induce type 1 T cell responses, corresponding to the type 1 cytokine expression patterns observed in progressive vitiligo [63].

Immune Regulation

Differential progression of the immune response among vitiligo and melanoma patients is also determined by regulatory T cells. Melanoma immunotherapy studies have demonstrated that depletion or suppression of regulatory T cells improves the induction of antimelanoma immune responses. Regulatory T cells apparently interfere with antitumor reactivity in melanoma patients, preventing a successful outcome of antitumor immunotherapy [68]. Indeed, melanoma tissue contains the immunosuppressive cytokines IL-10 and TGF- β (transforming growth factor beta), indicative of immunoregulation taking place within the tumor microenvironment.

Where melanocyte-specific T cells occasionally infiltrate the skin, local immune suppression by skin-resident regulatory T cells prohibits these autoreactive T cells from exerting effector functions in healthy individuals. Regulatory/suppressor T cells expressing immunosuppressive cytokines including IL-10 and TGF- β are continuously present in the skin, keeping ongoing immune responses in check [69]. The regulatory T cells thereby preserve skin homeostasis, which can be overruled in inflammatory conditions. In melanoma tissue, however, the level of immune suppression is more pronounced and impairs most of the effector function of antitumor immune responses. In addition, local immunosurveillance of the skin immune system against the outgrowth of melanoma cells, which is present in healthy individuals, is actively suppressed in melanoma patients, allowing tumor outgrowth.

Vitiligo patients generally lack effective immunoregulation, which allows high-avidity T cells to attack melanocytes in the skin. The association observed between vitiligo and other autoimmune diseases is indicative of this lack of immune regulation. In the microenvironment of the vitiligo skin, infiltrating T cells may undergo a process analogous to affinity maturation among B cells, resulting in T cells with TCR of increasing affinity, which cause progressive depigmentation. Recent data indicate that regulatory T cell function is altered in vitiligo, and in the absence of functional, skin-infiltrating regulatory T cells the ongoing immune response is perpetuated [unpubl. data]. Such intrinsic defect may also explain the marked difference in distribution patterns of the lesions in patients with autoimmune vitiligo in contrast to patients that develop progressive depigmentation secondary to their disease, as in melanoma.

Intervention

A definitive cure for vitiligo has yet to be found. Currently, successful therapeutic measures require a situation of stable disease to repopulate lesional vitiligo

Table 1. Current and future treatment options for vitiligo

Treatment step 1 (inhibiting depigmentation)	Principle mechanism
Detoxifying enzymes	Supporting the antioxidant defense in remaining melanocytes, thus preventing premature apoptosis
Corticosteroids/tacrolimus	Generic immunosuppressants
Cytokine inhibitors	Preventing immune activation by depleting cytokines required
Immature dendritic cells	Presenting antigens in the absence of costimulation, thus preventing T cell activation
Tuning with peptides	Deviating clonal selection in primary lymphoid organs by introducing artificial low-affinity proteins
Regulatory T cells	Adoptive transfer of T cells capable of inhibiting an ongoing immune response
MSH	Immunosuppressive properties
UVB	Immunosuppression in the exposed skin
Treatment step 2 (promoting repigmentation)	Principle mechanism
UVB	Stimulating melanogenesis, melanocyte proliferation and migration
Surgery	Covering depigmented areas with whole skin grafts, epidermis from suction blisters or cultured cells
MSH	Melanogenic, prolific and migratory stimulant for melanocytes

skin with melanocytes by a variety of surgical skin grafting methods in combination with (narrow-band) UVB radiation. The problem remains that patients with a propensity to develop vitiligo run the risk of recurrent disease, and curing vitiligo is clearly a two-tiered process consisting of halting progression of depigmentation followed by repigmentation of lesional skin. Effective measures are available to repopulate stable vitiligo lesions by surgical transplantation measures in combination with UVB treatment to stimulate melanocyte migration, proliferation and melanization [70]. The perceived inefficacy of available vitiligo treatments results from a lack of focus on halting disease progression, most notably by interfering with autoimmune reactivity to melanocytes. Several principal steps can be taken towards intervention, as summarized in table 1. In the end, a two-tiered approach is necessary to successfully treat vitiligo.

Acknowledgements

This work was supported in part by NIH/NCI grant RO1CA109536, NIH/NIAMS grant RO3AR050137 and support from the National Vitiligo Foundation (USA) to C.L.P.

R.M.L. is supported by grants from the Netherlands Organisation for Scientific Research (NWO-VIDI 016.056.337) and the Dutch Cancer Society (UVA2006-3606).

References

- 1 Birlea S, Birlea M, Cimponeriu D, Apostol P, Cosgarea R, Gavrilă L, Tigan S, Costin G, Das P: Autoimmune diseases and vitamin D receptor Apa-I polymorphism are associated with vitiligo in a small inbred Romanian community. *Acta Derm Venereol* 2006;86:209–214.
- 2 Mason CP, Gawkrödger DJ: Vitiligo presentation in adults. *Clin Exp Dermatol* 2005;30:344–345.
- 3 Agarwal G: Vitiligo: an under-estimated problem. *Fam Pract* 1998;15(suppl 1):S19–S23.
- 4 Dogra S, Parsad D, Handa S, Kanwar AJ: Late onset vitiligo: a study of 182 patients. *Int J Dermatol* 2005;44:193–196.
- 5 Hann SK, Lee HJ: Segmental vitiligo: clinical findings in 208 patients. *J Am Acad Dermatol* 1996;35: 671–674.
- 6 Ongenaes K, van Geel N, Naeyaert JM: Evidence for an autoimmune pathogenesis of vitiligo. *Pigment Cell Res* 2003;16:90–100.
- 7 Hartmann A, Brocker EB, Becker JC: Hypopigmentary skin disorders: current treatment options and future directions. *Drugs* 2004;64:89–107.
- 8 Kakourou T, Kanaka-Gantenbein C, Papadopoulou A, Kaloumenou E, Chrousos GP: Increased prevalence of chronic autoimmune (Hashimoto's) thyroiditis in children and adolescents with vitiligo. *J Am Acad Dermatol* 2005;53:220–223.
- 9 Harning R, Cui J, Bystryń JC: Relation between the incidence and level of pigment cell antibodies and disease activity in vitiligo. *J Invest Dermatol* 1991;97:1078–1080.
- 10 Gottumukkala RV, Gavalas NG, Akhtar S, Metcalfe RA, Gawkrödger DJ, Haycock JW, Watson PF, Weetman AP, Kemp EH: Function-blocking autoantibodies to the melanin-concentrating hormone receptor in vitiligo patients. *Lab Invest* 2006;86:781–789.
- 11 van den Wijngaard RM, Asghar SS, Pijnenborg AC, Tigges AJ, Westerhof W, Das PK: Aberrant expression of complement regulatory proteins, membrane cofactor protein and decay accelerating factor, in the involved epidermis of patients with vitiligo. *Br J Dermatol* 2002;146:80–87.
- 12 Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK: Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am J Pathol* 1996;148: 1219–1228.
- 13 van den Wijngaard RM, Wankowicz-Kalinska A, Le Poole C, Tigges B, Westerhof W, Das P: Local immune response in skin of generalized vitiligo patients: destruction of melanocytes is associated with the prominent presence of CLA+ T cells at the perilesional site. *Lab Invest* 2000;80: 1299–1309.
- 14 Lang KS, Caroli CC, Muhm A, Wernet D, Moris A, Schitteck B, Knauss-Scherwitz E, Stevanovic S, Rammensee HG, Garbe C: HLA-A2 restricted, melanocyte-specific CD8+ T lymphocytes detected in vitiligo patients are related to disease activity and are predominantly directed against MelanA/MART1. *J Invest Dermatol* 2001;116:891–897.
- 15 Mandelcorn-Monson RL, Shear NH, Yau E, Sambhara S, Barber BH, Spaner D, DeBenedette MA: Cytotoxic T lymphocyte reactivity to gp100, MelanA/MART-1, and tyrosinase, in HLA-A2-positive vitiligo patients. *J Invest Dermatol* 2003;121:550–556.
- 16 Ogg GS, Rod DP, Romero P, Chen JL, Cerundolo V: High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. *J Exp Med* 1998;188:1203–1208.
- 17 Palermo B, Campanelli R, Garbelli S, Mantovani S, Lantelme E, Brazzelli V, Ardigò M, Borroni G, Martinetti M, Badulli C, Necker A, Giachino C: Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. *J Invest Dermatol* 2001;117:326–332.
- 18 Wang X, Erf GF: Melanocyte-specific cell mediated immune response in vitiliginous Smyth line chickens. *J Autoimmun* 2003;21:149–160.

- 19 Kawakami Y, Dang N, Wang X, Tupesis J, Robbins PF, Wang RF, Wunderlich JR, Yannelli JR, Rosenberg SA: Recognition of shared melanoma antigens in association with major HLA-A alleles by tumor infiltrating T lymphocytes from 123 patients with melanoma. *J Immunother* 2000;23: 17–27.
- 20 Bystryn JC, Rigel D, Friedman RJ, Kopf A: Prognostic significance of hypopigmentation in malignant melanoma. *Arch Dermatol* 1987;123:1053–1055.
- 21 Merimsky O, Shoenfeld Y, Yecheskel G, Chaichik S, Azizi E, Fishman P: Vitiligo- and melanoma-associated hypopigmentation: a similar appearance but a different mechanism. *Cancer Immunol Immunother* 1994;38:411–416.
- 22 Nordlund JJ, Kirkwood JM, Forget BM, Milton G, Albert DM, Lerner AB: Vitiligo in patients with metastatic melanoma: a good prognostic sign. *J Am Acad Dermatol* 1983;9:689–696.
- 23 Gogas H, Ioannovich J, Dafni U, Stavropoulou-Giokas C, Frangia K, Tsoutsos D, Panagiotou P, Polyzos A, Papadopoulos O, Stratigos A, Markopoulos C, Bafaloukos D, Pectasides D, Fountzilias G, Kirkwood JM: Prognostic significance of autoimmunity during treatment of melanoma with interferon. *N Engl J Med* 2006;354:709–718.
- 24 Becker JC, Guldberg P, Zeuthen J, Brocker EB, Straten PT: Accumulation of identical T cells in melanoma and vitiligo-like leukoderma. *J Invest Dermatol* 1999;113:1033–1038.
- 25 Kiniwa Y, Fujita T, Akada M, Ito K, Shofuda T, Suzuki Y, Yamamoto A, Saida T, Kawakami Y: Tumor antigens isolated from a patient with vitiligo and T-cell-infiltrated melanoma. *Cancer Res* 2001;61:7900–7907.
- 26 Le Gal FA, Avril MF, Bosq J, Lefebvre P, Deschemin JC, Andrieu M, Dore MX, Guillet JG: Direct evidence to support the role of antigen-specific CD8+ T cells in melanoma-associated vitiligo. *J Invest Dermatol* 2001;117:1464–1470.
- 27 Luiten RM, Kueter EW, Mooi W, Gallee MP, Rankin EM, Gerritsen WR, Clift SM, Nooijen WJ, Weder P, van de Kastele WF, Sein J, van den Berk PC, Nieweg OE, Berns AM, Spits H, de Gast GC: Immunogenicity, including vitiligo, and feasibility of vaccination with autologous GM-CSF-transduced tumor cells in metastatic melanoma patients. *J Clin Oncol* 2005;23:8978–8991.
- 28 Merimsky O, Shoenfeld Y, Baharav E, Zigelman R, Fishman P: Reactivity to tyrosinase: expression in cancer (melanoma) and autoimmunity (vitiligo). *Hum Antibodies Hybridomas* 1996;7: 151–156.
- 29 Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, Rosenberg SA: Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 2002;298:850–854.
- 30 Jager E, Maeurer M, Hohn H, Karbach J, Jager D, Zidianakis Z, Bakhshandeh-Bath A, Orth J, Neukirch C, Necker A, Reichert TE, Knuth A: Clonal expansion of Melan A-specific cytotoxic T lymphocytes in a melanoma patient responding to continued immunization with melanoma-associated peptides. *Int J Cancer* 2000;86:538–547.
- 31 Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, Duray PH, Steinberg SM, Allison JP, Davis TA, Rosenberg SA: Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci USA* 2003;100:8372–8377.
- 32 Tsao H, Millman P, Linette GP, Hodi FS, Sober AJ, Goldberg MA, Haluska FG: Hypopigmentation associated with an adenovirus-mediated gp100/MART-1-transduced dendritic cell vaccine for metastatic melanoma. *Arch Dermatol* 2002;138:799–802.
- 33 Yee C, Thompson JA, Roche P, Byrd DR, Lee PP, Piepkorn M, Kenyon K, Davis MM, Riddell SR, Greenberg PD: Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of t cell-mediated vitiligo. *J Exp Med* 2000;192:1637–1644.
- 34 Au WY, Yeung CK, Chan HH, Lie AK: Generalized vitiligo after lymphocyte infusion for relapsed leukaemia. *Br J Dermatol* 2001;145:1015–1017.
- 35 Palermo B, Garbelli S, Mantovani S, Scoccia E, Da Prada GA, Bernabei P, Avanzini MA, Brazzelli V, Borroni G, Giachino G: Qualitative difference between the cytotoxic T lymphocyte responses to melanocyte antigens in melanoma and vitiligo. *Eur J Immunol* 2005;35:3153–3162.

- 36 van den Boorn JG, Le Poole IC, Luiten RM: T-cell avidity and tuning: the flexible connection between tolerance and autoimmunity. *Int Rev Immunol* 2006;25:235–258.
- 37 Mantovani S, Garbelli S, Palermo B, Campanelli R, Brazzelli V, Borroni G, Martinetti M, Benvenuto F, Merlini G, della Cuna GR, Rivoltini L, Giachino C: Molecular and functional bases of self-antigen recognition in long-term persistent melanocyte-specific CD8+ T cells in one vitiligo patient. *J Invest Dermatol* 2003;121:308–314.
- 38 Cui J, Chen D, Misfeldt ML, Swinfard RW, Bystryń JC: Antimelanoma antibodies in swine with spontaneously regressing melanoma. *Pigment Cell Res* 1995;8:60–63.
- 39 Engelhard VH, Bullock TN, Colella TA, Sheasley SL, Mullins DW: Antigens derived from melanocyte differentiation proteins: self-tolerance, autoimmunity, and use for cancer immunotherapy. *Immunol Rev* 2002;188:136–146.
- 40 Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, de Jong LA, Vyth-Dreese FA, Dellemijn TA, Antony PA, Spiess PJ, Palmer DC, Heimann DM, Klebanoff CA, Yu Z, Hwang LN, Feigenbaum L, Kruisbeek AM, Rosenberg SA, Restifo NP: Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. *J Exp Med* 2003;198:569–580.
- 41 Suttmüller RP, van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, Toes RE, Offringa R, Melief CJ: Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25+ regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* 2001;194:823–832.
- 42 Overwijk WW, Lee DS, Surman DR, Irvine KR, Touloukian CE, Chan CC, Carroll MW, Moss B, Rosenberg SA, Restifo NP: Vaccination with a recombinant vaccinia virus encoding a ‘self’ antigen induces autoimmune vitiligo and tumor cell destruction in mice: requirement for CD4+ T lymphocytes. *Proc Natl Acad Sci USA* 1999;96:2982–2987.
- 43 Hasse S, Gibbons NC, Rokos H, Marles LK, Schallreuter KU: Perturbed 6-tetrahydrobiopterin recycling via decreased dihydropteridine reductase in vitiligo: more evidence for H₂O₂ stress. *J Invest Dermatol* 2004;122:307–313.
- 44 Park B, Lee S, Kim E, Cho K, Riddell SR, Cho S, Ahn K: Redox regulation facilitates optimal peptide selection by MHC class I during antigen processing. *Cell* 2006;127:369–382.
- 45 Tsatmali M, Ancans J, Thody AJ: Melanocyte function and its control by melanocortin peptides. *J Histochem Cytochem* 2002;50:125–133.
- 46 Pichler R, Sfetos K, Badics B, Gutenbrunner S, Aubock J: Vitiligo patients present lower plasma levels of alpha-melanotropin immunoreactivities. *Neuropeptides* 2006;40:177–183.
- 47 Spencer JD, Gibbons NC, Rokos H, Peters EM, Wood JM, Schallreuter KU: Oxidative stress via hydrogen peroxide affects proopiomelanocortin peptides directly in the epidermis of patients with vitiligo. *J Invest Dermatol* 2007;127:411–420.
- 48 Luger TA, Scholzen TE, Brzoska T, Böhm M: New insights into the functions of α-MSH and related peptides in the immune system. *Ann NY Acad Sci* 2003;994:133–140.
- 49 Milani V, Noessner E, Ghose S, Kuppler M, Ahrens B, Scharner A, Gastpar R, Issels RD: Heat shock protein 70: role in antigen presentation and immune stimulation. *Int J Hyperthermia* 2002;18:563–575.
- 50 Boissy RE, Manga P: On the etiology of contact/occupational vitiligo. *Pigment Cell Res* 2004;17:208–214.
- 51 Mosher DB, Parrish JA, Fitzpatrick TB: Monobenzylether of hydroquinone: a retrospective study of treatment of 18 vitiligo patients and a review of the literature. *Br J Dermatol* 1977;97:669–679.
- 52 Ivanova K, Le Poole IC, Gerzer R, Westerhof W, Das PK: Effect of nitric oxide on the adhesion of human melanocytes to extracellular matrix components. *J Pathol* 1997;183:469–476.
- 53 Iuga AO, Qureshi AA, Lerner EA: Nitric oxide is toxic to melanocytes in vitro. *Pigment Cell Res* 2004;17:302–306.
- 54 Huang CL, Nordlund JJ, Boissy R: Vitiligo: a manifestation of apoptosis? *Am J Clin Dermatol* 2002;3:301–308.
- 55 Swope VB, Supp AP, Schwemberger S, Babcock G, Boyce S: Increased expression of integrins and decreased apoptosis correlate with increased melanocyte retention in cultured skin substitutes. *Pigment Cell Res* 2006;19:424–433.

- 56 Kroll TM, Bommasamy H, Boissy RE, Hernandez C, Nickoloff BJ, Mestrl R, Caroline LP, I: 4-Tertiary butyl phenol exposure sensitizes human melanocytes to dendritic cell-mediated killing: relevance to vitiligo. *J Invest Dermatol* 2005;124:798–806.
- 57 Le Poole IC, Mutis T, van den Wijngaard RM, Westerhof W, Ottenhoff T, de Vries RR, Das PK: A novel, antigen-presenting function of melanocytes and its possible relationship to hypopigmentary disorders. *J Immunol* 1993;151:7284–7292.
- 58 Kristiansen OP, Larsen ZM, Pociot F: CTLA-4 in autoimmune diseases: a general susceptibility gene to autoimmunity? *Genes Immun* 2000;1:170–184.
- 59 Chakraborty AK, Sousa JF, Chakraborty D, Funasaka Y, Bhattacharya M, Chatterjee A, Pawelek J: GnT-V expression and metastatic phenotypes in macrophage-melanoma fusion hybrids is down-regulated by 5-Aza-dC: evidence for methylation sensitive, extragenic regulation of GnT-V transcription. *Gene* 2006;374:166–173.
- 60 Byrne SN, Halliday GM: Phagocytosis by dendritic cells rather than MHC II^{high} macrophages is associated with skin tumour regression. *Int J Cancer* 2003;106:736–744.
- 61 Kao CH, Yu HS: Depletion and repopulation of Langerhans cells in nonsegmental type vitiligo. *J Dermatol* 1990;17:287–296.
- 62 Le Poole IC, Stennett LS, Bonish BK, Dee L, Robinson JK, Hernandez C, Hann SK, Nickoloff BJ: Expansion of vitiligo lesions is associated with reduced epidermal CDw60 expression and increased expression of HLA-DR in perilesional skin. *Br J Dermatol* 2003;149:739–748.
- 63 Wankowicz-Kalinska A, van den Wijngaard RM, Tigges BJ, Westerhof W, Ogg GS, Cerundolo V, Storkus WJ, Das PK: Immunopolarization of CD4⁺ and CD8⁺ T cells to Type-1-like is associated with melanocyte loss in human vitiligo. *Lab Invest* 2003;83:683–695.
- 64 Pittet MJ, Zippelius A, Valmori D, Speiser DE, Cerottini JC, Romero P: Melan-A/MART-1-specific CD8 T cells: from thymus to tumor. *Trends Immunol* 2002;23:325–328.
- 65 Collins SM, Dominguez M, Ilmarinen T, Costigan C, Irvine AD: Dermatological manifestations of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome. *Br J Dermatol* 2006;154:1088–1093.
- 66 Coutinho A, Caramalho I, Seixas E, Demengeot J: Thymic commitment of regulatory T cells is a pathway of TCR-dependent selection that isolates repertoires undergoing positive or negative selection. *Curr Top Microbiol Immunol* 2005;293:43–71.
- 67 Matzinger P: The danger model: a renewed sense of self. *Science* 2002;296:301–305.
- 68 Turk MJ, Guevara-Patino JA, Rizzuto GA, Engelhorn ME, Sakaguchi S, Houghton AN: Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells. *J Exp Med* 2004;200:771–782.
- 69 Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK: Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev* 2006;212:28–50.
- 70 Pianigiani E, Risulo M, Andreassi A, Taddeucci P, Ierardi F, Andreassi L: Autologous epidermal cultures and narrow-band ultraviolet B in the surgical treatment of vitiligo. *Dermatol Surg* 2005;31:155–159.

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The Genetics of Generalized Vitiligo

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Abstract

Generalized vitiligo is an acquired disorder in which patches of depigmented skin, overlying hair and oral mucosa result from progressive autoimmune loss of melanocytes from the involved areas. Perhaps the most common pigmentary disorder, vitiligo results from a complex interaction of environmental, genetic and immunologic factors that ultimately contribute to melanocyte destruction, resulting in the characteristic depigmented lesions. In the past few years, studies of the genetic epidemiology of generalized vitiligo have led to the recognition that vitiligo is part of a broader, genetically determined, autoimmune and autoinflammatory diathesis. Attempts to identify genes involved in vitiligo susceptibility have involved gene expression studies, allelic association studies of candidate genes and genome-wide linkage analyses to discover new genes, and these studies have begun to shed light on the mechanisms of vitiligo pathogenesis. It is anticipated that the discovery of biological pathways of vitiligo pathogenesis will provide novel therapeutic and prophylactic targets for future approaches to the treatment and prevention of vitiligo and its associated autoimmune diseases.

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Generalized vitiligo is an acquired, noncontagious disorder in which progressive, patchy loss of pigmentation from skin, overlying hair and oral mucosa (fig. 1) results from loss of melanocytes from the involved areas [1, 2]. Known for thousands of years because of its visually evident phenotype, vitiligo is perhaps the most common pigmentary disorder, affecting about 0.38% of Caucasians [3] and occurring with a generally similar frequency in other populations throughout the world [4, 5]. Many different etiologic hypotheses have been suggested for vitiligo [1, 2], the most compelling of which involve a combination of environmental, genetic and immunologic factors interacting to contribute to autoimmune melanocyte destruction. Nevertheless, the specific causes of generalized vitiligo remain obscure and no common environmental factors that trigger generalized vitiligo, either directly or via an autoimmune response,



Fig. 1. A patient with generalized vitiligo. Note obvious patches of white skin in typical distribution involving the periorbital region and hands.

have yet been identified with certainty. This limited progress has resulted, in large part, from the lack of a clear definition of the disorder and the lack of a tractable experimental animal model of typical human generalized vitiligo that can be manipulated and studied in the laboratory.

In recent years, technological advances enabled by the Human Genome Project, and methodological advances applied to analyses of polygenic multifactorial diseases have led to efforts to map and identify specific genes involved in vitiligo susceptibility and pathogenesis. As a result, there has been considerable recent progress towards the identification of vitiligo susceptibility genes, some of which may provide novel therapeutic and prophylactic targets for new interventional approaches to treat and even prevent vitiligo in the future.

Genetic Epidemiology of Generalized Vitiligo

Recent progress in defining the genetic underpinnings of vitiligo has hinged on clearly defining the disorder, thus permitting investigators to test specific hypotheses via carefully controlled studies. Accordingly, most studies have focused on generalized vitiligo. Generalized vitiligo is defined as an acquired pigmentary disorder characterized by depigmentation due to melanocyte loss in

the regions of involved skin, in a pattern that is nonfocal and generally bilateral across the midline, though not necessarily symmetric [6]. This definition thus excludes various Mendelian hypopigmentary spotting disorders, such as piebaldism and the various forms of Waardenburg syndrome, which result from mutations in specific single genes and which are characterized by congenital white spotting that is relatively stable over patients' lifetimes [7]. This definition of generalized vitiligo also excludes segmental vitiligo and some other localized forms of vitiligo, whose true pathogenic relationship to generalized vitiligo remains unknown, but includes acrofacial vitiligo, which often progresses to more extensive skin involvement and, like classic generalized vitiligo, is often associated with other autoimmune and autoinflammatory disorders. The strict definition of generalized vitiligo also excludes many other forms of skin depigmentation, such as depigmentation resulting from contact or occupational exposure to known depigmenting agents, including phenols, catechols, quinines and other compounds, depigmentation secondary to chronic inflammation, psoriasis, other forms of dermatitis, and depigmentation secondary to infection, scars, burns and various other skin insults. This is not to say that these other forms of depigmentation might not share some biological pathways of disease pathogenesis with generalized vitiligo, but strictly defined, they are not vitiligo.

Large-scale epidemiologic surveys have shown that most cases of generalized vitiligo occur sporadically, although about 15–20% of patients have one or more affected first-degree relatives. Very rarely, large multigeneration families segregate vitiligo in an autosomal dominant pattern with incomplete penetrance [8]. More typically, however, familial aggregation of generalized vitiligo cases takes a non-Mendelian pattern that is suggestive of polygenic, multifactorial inheritance [4, 9–18]. Nevertheless, most vitiligo patients have no known family history of the disorder.

Strongest evidence for genetic factors in the pathogenesis of generalized vitiligo comes from studies of patients' close relatives. Among Caucasians, the risk to a patient's siblings is about 6.1% [17], a 16-fold increased risk (termed λ_s) over the approximately 0.38% prevalence of generalized vitiligo in the Caucasian population [3]. There is a similar risk of generalized vitiligo to patients' other first-degree relatives besides siblings: 7.1% in Caucasians, 6.1% in Indo-Pakistanis and 4.8% in Hispanics [17], with lower risks to more distant relatives. Similar results have come from studies of Chinese families [18]. Additional evidence for a genetic component to generalized vitiligo comes from age-of-onset data: among unselected (mostly sporadic) Caucasian vitiligo patients, the mean age of disease onset is 24.2 years [17], but among patients in multiplex families, in which multiple family members are affected by vitiligo, the mean age of disease onset is significantly earlier at 21.5 years [19]. These observations, of earlier disease onset in familial cases and decreased disease

risk with increasing genetic distance from an affected proband, are typical characteristics of a polygenic disorder, and formal genetic segregation analyses of vitiligo have suggested that multiple major loci contribute to vitiligo susceptibility in a complex interactive manner [15, 16, 18]. These and other data thus indicate that genetic factors are of considerable importance in determining one's susceptibility to generalized vitiligo.

Nevertheless, twin studies have shown that, although genetics plays an important role in the pathogenesis of generalized vitiligo, nongenetic factors must also be extremely important, perhaps even more important than genetic factors. In the largest vitiligo twin study to date [17], the concordance for generalized vitiligo in monozygotic twins was 23%. This is more than 60 times the general population risk of 0.38% and almost 4 times the 6.1% risk of vitiligo to probands' siblings, thus strongly supporting the importance of genes in one's risk for generalized vitiligo. However, identical twins share all of their genes identically, and the limited concordance thus also indicates that nongenetic, presumably environmental factors must also play a major role. Unfortunately, although many different environmental risk factors for generalized vitiligo have been proposed, as yet there really are no compelling scientific data supporting the involvement of any of these factors in the pathogenesis of generalized vitiligo in humans.

Genetic Association of Generalized Vitiligo with Other Autoimmune Diseases

Melanocyte loss in generalized vitiligo is now widely thought to occur on an autoimmune basis [1, 20–22], although the triggers and specific nature of the autoimmune response remain unknown. Circulating antibodies to melanocytes and various melanocytic protein components are detectable in many but not all patients, although most investigators consider these humoral responses to melanocyte destruction rather than a primary cause [23]. Of greater importance may be the occurrence of circulating skin-homing melanocyte-specific cytotoxic T lymphocytes [24] and sparse infiltrates of activated and cytotoxic T cells at the margins of active lesions [25–27], although the fraction of patients with such infiltrates is unclear [20, 21].

Perhaps the strongest evidence for an autoimmune origin of generalized vitiligo is its close epidemiological association with other autoimmune and autoinflammatory diseases. Generalized vitiligo is a component of the APECED (APS1) and Schmidt (APS2) multiple autoimmune disease syndromes, and in several studies vitiligo has been associated with autoimmune thyroid disease [28, 29], pernicious anemia [30, 31], Addison's disease [32] and perhaps alopecia areata [33, 34].

A survey of more than 2,600 unselected Caucasian patients with generalized vitiligo (most with sporadic occurrence of the disease) and their close relatives [17] found significantly increased frequencies of autoimmune thyroid disease, pernicious anemia, Addison's disease and systemic lupus erythematosus. Overall, about 30% of patients with generalized vitiligo were affected with at least one additional autoimmune or autoinflammatory disorder. These same vitiligo-associated autoimmune and autoinflammatory diseases also occurred at increased prevalence in patients' first-degree relatives, regardless of whether or not those relatives had vitiligo themselves [17]. Together, the findings indicated that vitiligo patients and their close relatives have a genetically determined susceptibility to this specific group of autoimmune and autoinflammatory diseases. A similar study of families in which multiple individuals had generalized vitiligo found even higher frequencies of these same autoimmune and inflammatory diseases, in both vitiligo patients and their siblings, as well as significantly elevated frequencies of psoriasis, rheumatoid arthritis and adult-onset autoimmune diabetes mellitus [19]. Such multiplex generalized vitiligo families thus exhibit an expanded repertoire of vitiligo-associated autoimmune and autoinflammatory diseases, indicating that these families segregate even greater genetic susceptibility to autoimmune and autoinflammatory diseases than singleton patients. Generally, similar results have come from retrospective studies of vitiligo patients in India [35, 36] and Nigeria [37], although these studies generally found lower frequencies of certain vitiligo-associated autoimmune diseases, most likely due to under-diagnosis of these autoimmune diseases in these populations. Together, these studies indicate that pathologic variants in specific genes predispose to a distinct group of autoimmune diseases that includes generalized vitiligo, autoimmune thyroid disease, rheumatoid arthritis, psoriasis, adult-onset autoimmune diabetes mellitus, pernicious anemia, systemic lupus erythematosus and Addison's disease (fig. 2). As will be discussed below, several of these broad-spectrum autoimmunity genes have now been identified. Nevertheless, additional vitiligo susceptibility genes and/or environmental triggers are certainly involved in susceptibility to specific autoimmune diseases in most cases.

Identification of Vitiligo Susceptibility Genes

Three very different approaches have been used to identify genes that mediate susceptibility to vitiligo (table 1). Gene expression analyses have attempted to identify genes that are differentially expressed in cells or tissue from vitiligo patients versus controls, or from disease tissue versus normal tissue from patients. These studies can generate lists of candidate genes, but they cannot distinguish genes with primary effects from the many more genes whose expression

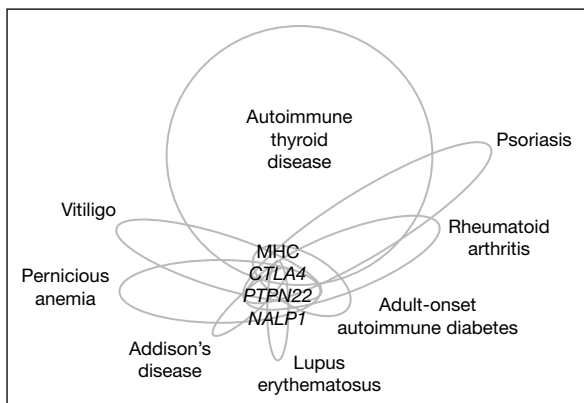


Fig. 2. Vitiligo-associated autoimmune and autoinflammatory disease diathesis.

may be dysregulated on a secondary basis that show differential expression because of individual variation due to the outbred genetic background among humans. Genetic association studies have been used to test specific candidate genes, considered to perhaps be involved in vitiligo susceptibility on the basis of an a priori biological hypothesis. This approach can be used to study singleton patients, who represent the majority of cases. However, association analyses can be highly subject to both false-positive and false-negative errors due to population admixture and population differences. Furthermore, the genetic association approach has been limited to testing already known candidate genes. Genetic linkage studies, by contrast, can be used to scan the entire human genome for chromosomal regions that nonrandomly segregate with vitiligo in multiplex families. The linkage approach has the advantage that it can identify entirely novel genes that were previously unsuspected, but it is limited to analysis of multiplex families, whose genetic underpinnings may not be quite the same as in the predominant singleton cases. Indeed, thus far there has been little correlation between the results obtained by these two different approaches to identifying vitiligo susceptibility genes. Genome-wide association is a new approach that offers the benefits of both the genetic linkage and association approaches, but has not yet been applied to study the genetics of vitiligo.

Gene Expression Studies

VIT1 is a gene located at chromosome 2p21 (previously assigned to 2p16), and was originally named on the basis of its apparent aberrant expression in

Table 1. Candidate genes suggested for involvement in vitiligo

Chromosome	Gene or locus	Method	Comments
1p31.3-p32.2	<i>AIS1 (FOXD3?)</i>	Linkage, positional cloning	Rare autosomal dominant; atypical phenotype, autoimmunity-associated
1p13	<i>PTPN22</i>	Association	Associated with many autoimmune disorders
2p21	<i>VIT1 (FBXO11)</i>	Expression analysis	No evidence for causal involvement in vitiligo
2q33	<i>CTLA4</i>	Association	Associated with many autoimmune disorders; data conflicting
3p14.1-p12.3	<i>MITF</i>	Linkage	No evidence for linkage
6p21.3	MHC (<i>HLA-DRB1</i> , <i>HLA-DRB4</i> , <i>HLA-DQB1</i>)	Association, linkage	Associated with many autoimmune disorders
6q25.1	<i>ESR</i>	Association	Autoimmunity-associated
7	<i>AIS2</i>	Linkage	
8	<i>AIS3</i>	Linkage	
10q11.2-q21	<i>MBL2</i>	Association	
11p13	<i>CAT</i>	Association	Now considered invalid
12q12-q14	<i>VDR</i>	Association	
12q13	<i>MYG1</i>	Expression analysis	Now considered invalid
14q22.1-q22.2	<i>GCHI</i>	Association and sequencing	
17p13	<i>NALP1 (SLEVI)</i>	Linkage and association	Autoimmunity associated
17q23	<i>ACE</i>	Association	Data conflicting
21q22.3	<i>AIRE</i>	Linkage and sequencing	Autoimmunity associated (causes autosomal recessive APECED syndrome); no association in typical vitiligo
22q11.2	<i>COMT</i>	Association	

intralesional vitiligo melanocytes [38]. *VIT1* is now officially named *FBXO11*. Similarly, *MYG1*, a widely expressed gene located at chromosome 12q13, was shown on the basis of differential hybridization to have elevated expression in melanocytes from vitiligo patients [39]. However, there is no evidence that either *FBXO11* or *MYG1* are causally involved in the pathogenesis of vitiligo.

Genetic Association Studies

The earliest genetic studies of vitiligo were case-control allelic association studies of the major histocompatibility complex (MHC), carried out by testing various different MHC markers in patients with various different vitiligo phenotypes versus controls, from many different populations [40–50]. In general, these studies have found no consistent association between the occurrence of vitiligo and specific HLA alleles. However, reanalysis of these studies as a group shows that several found association between vitiligo and *HLA-DRB4* alleles, and meta-analysis found association of vitiligo with *HLA-A2* [50]. Recent studies that have used more robust and modern statistical methods found association of generalized vitiligo with *HLA-DRB4*0101* and *HLA-DQB1*0303* in Dutch patients [46], with *HLA-DRB1*03*, *HLA-DRB1*04* and *HLA-DRB1*07* alleles in Turkish patients [48], and with alleles of microsatellites located in the MHC in Columbian patients [47]. In Caucasian multiplex generalized vitiligo families, the MHC class II haplotype *HLA-DRB1A*04-(DQA1*0302)-DQB1*0301* is associated with both increased risk of vitiligo and relatively early disease onset [51], and in Han Chinese the MHC haplotype *HLA-A25-Cw*0602-DQA1*0302* is associated with generalized vitiligo [50]. Allelic association has also been reported between generalized vitiligo and genes of the *LMP/TAP* gene region of the MHC [52], although the significance of this is, as yet, unclear. Genetic associations of vitiligo with alleles of MHC loci appear to be strongest in patients and families with various vitiligo-associated autoimmune/autoinflammatory disorders, versus patients and families with only generalized vitiligo. Many of these nonvitiligo autoimmune/autoinflammatory diseases are themselves associated with variation in the MHC, and it remains uncertain whether these reported vitiligo-MHC associations are primary or instead actually represent MHC association with these other diseases. Indeed, genome-wide genetic linkage analyses of vitiligo have shown no apparent linkage signal at the MHC in Caucasians, although a minor linkage signal in this region of chromosome 6 has been reported in Han Chinese [53].

Allelic associations between vitiligo and a number of other candidate genes on other chromosomes have also been described. A reported association

between vitiligo/dopa-responsive dystonia and the GTP-cyclohydrolase (*GCHI*) gene [54] was subsequently shown to be spurious [55]. Likewise, reported allelic association between vitiligo and the catalase (*CAT*) gene [56] was essentially refuted by subsequent studies [57, 58]. Similarly, reported association between vitiligo and variation in the gene encoding angiotensin-converting enzyme (*ACE*) [59] were not confirmed in another study [60], albeit patients were from a different population. Allelic associations have also been reported between vitiligo and the estrogen receptor 1 (*ESR1*) [61], catechol-O-methyltransferase (*COMT*) [62] and vitamin D receptor (*VDR*) [63] genes. These associations have not yet been confirmed, and several are based on marginally significant results and seem of dubious validity.

Three other allelic associations have been reported between generalized vitiligo and genes that are plausibly implicated in the autoimmune process: *CTLA4* [64], *PTPN22* [65] and *MBL2* [66]. While these associations remain to be confirmed, variations in *CTLA4* and *PTPN22* have been broadly implicated in a number of other autoimmune diseases, and these genes may, like HLA, function as general autoimmunity/autoinflammatory susceptibility loci [67].

Genetic Linkage Studies

The first vitiligo linkage data were negative, showing lack of apparent linkage to a single candidate gene tested, *MITF* [68]. The first positive linkage data were indirect, showing linkage between a locus on chromosome 17p13, called *SLEV1*, and systemic lupus erythematosus in families that included at least one patient with vitiligo [69].

The first genome-wide linkage analysis of vitiligo per se investigated a large, multigeneration family with generalized vitiligo and other vitiligo-associated autoimmune/autoinflammatory diseases, inherited as an apparent autosomal dominant trait with incomplete penetrance. Vitiligo in this family was mapped to a locus termed *AISI*, located in chromosome segment 1p31.3-p32.2, whereas susceptibility to other autoimmune/autoinflammatory disorders in the context of inheriting an *AISI* mutation was mapped to a region of chromosome 6 that included the MHC [8]. Detailed studies of genes in the *AISI* region of chromosome 1p in this family identified a promoter variant in *FOXD3*, a gene encoding an embryonic transcription factor that is a primary regulator of melanoblast differentiation and development. The promoter variant in this family increases transcriptional activity in transfected permissive cells by 50%, possibly interfering with the melanoblast/melanocyte differentiation or survival program, somehow predisposing to vitiligo [70]. The generalized vitiligo phenotype in this family is unusual, consisting of progressively coalescent skin

mottling which, in the context of autosomal dominant inheritance, make this family essentially unique. Indeed, other generalized vitiligo patients do not appear to have mutations of *FOXD3* and do not show linkage to the *AIS1* region of chromosome 1p.

Genome-wide linkage analyses of smaller multiplex families with clinically more typical generalized vitiligo have yielded linkage signals that may reflect additional novel susceptibility genes (table 1). In Caucasians, significant vitiligo linkage signals have been detected on chromosomes 7 (*AIS2*), 8 (*AIS3*) and 17p, and indications of additional suggestive linkage signals have been found on several other chromosomes [71, 72]. The chromosome 7 and 17p linkages appear to derive primarily from families with other, vitiligo-associated autoimmune/ autoinflammatory diseases, whereas the chromosome 8 linkage signal may reflect vitiligo per se [72]. In Chinese families with generalized vitiligo, genetic linkage studies of generalized vitiligo have detected an entirely different set of linkage signals [53], particularly on chromosome 4q13-q21, but also including signals at 1p36, 6p21-p22, 6q24-q25, 14q12-q13 and 22q12, none of which align with the linkages observed in Caucasian families, thus suggesting that different genes may be involved in the pathogenesis of vitiligo in different populations around the world. In general, these various vitiligo linkage signals do not appear to correspond to any of the various candidate genes that have been suggested for vitiligo, with the possible exception of *KIT* on 4q12 and the MHC on 6p21-p22.

Very recently, the vitiligo susceptibility gene on chromosome 17p has been identified, providing important insights into both vitiligo pathogenesis and possible new approaches to treatment [73]. The 17p vitiligo linkage signal, detected in families with a variety of vitiligo-associated autoimmune and autoinflammatory diseases [72], coincided with the location of *SLEVI*, a linkage signal originally detected in multiplex lupus families that included at least one case of vitiligo [69] and subsequently confirmed in other lupus families with various other autoimmune diseases [74]. Together, these findings suggested that 17p harbors at least one gene that mediates susceptibility to the entire repertoire of vitiligo-associated autoimmune and autoinflammatory diseases. Association analysis across the 17p linkage region in the vitiligo families used for linkage and subsequently confirmed in a second set of vitiligo families, identified *NALP1* as a major susceptibility gene for generalized vitiligo and the other autoimmune and autoinflammatory diseases associated with vitiligo [73], and this association has subsequently been confirmed by study of an independent patient cohort [75]. *NALP1* encodes a key regulator of the innate immune system that stimulates the interleukin-1 β inflammatory and apoptotic pathways in response to unknown bacterial or viral triggers. These findings suggest that drugs that modulate this pathways may offer new

approaches to treatment of generalized vitiligo, and perhaps others of these associated diseases.

Generalized vitiligo thus appears to be an autoimmune disease of multifactorial origin that results from a combination of multiple inherited genetic risk factors and environmental triggers. Several candidate genes and genetic linkages have been identified that appear to mediate susceptibility to both generalized vitiligo and to a specific group of other autoimmune and autoinflammatory disorders with which vitiligo is epidemiologically associated, including autoimmune thyroid disease, rheumatoid arthritis, psoriasis, adult-onset autoimmune diabetes mellitus, pernicious anemia, systemic lupus erythematosus and Addison's disease. Additional genes may mediate susceptibility to vitiligo itself. Identification of these genes will likely result in identification of biological pathways that mediate disease pathogenesis, providing novel interventional targets for both treatment and prevention of these disorders in genetically susceptible individuals.

Acknowledgements

This work was supported by grants AR45584 and AI46374 from the National Institutes of Health.

References

- 1 Nordlund JJ, Ortonne J-P, LePoole IC: Vitiligo vulgaris; in Nordlund JJ, Boissy RE, Hearing VJ, King RA, Oetting WS, Ortonne J-P (eds): *The Pigmentary System: Physiology and Pathophysiology*, ed 2. Blackwell, Malden, 2006, pp 551–598.
- 2 Hann S-K, Nordlund J: *Vitiligo*. Oxford, Blackwell Science, 2000.
- 3 Howitz J, Brodthagen H, Schwartz M, Thompsen K: Prevalence of vitiligo: epidemiological survey of the Isle of Bornholm, Denmark. *Arch Dermatol* 1977;113:47–52.
- 4 Das SK, Majumder PP, Chakraborty R, Majumdar TK, Halder B: Studies on vitiligo. I. Epidemiological profile in Calcutta, India. *Genet Epidemiol* 1985;2:71–78.
- 5 Lu T, Gao T, Wang A, Jin Y, Li Q, Li C: Vitiligo prevalence study in Shaanxi Province, China. *Int J Dermatol* 2007;46:47–51.
- 6 Taieb A, Picardo M; VETF Members: The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. *Pigment Cell Res* 2007;20:27–35.
- 7 Spritz RA, Chiang P-W, Oiso N, Alkhateeb A: Human and mouse disorders of pigmentation. *Curr Opin Genet Dev* 2003;13:284–289.
- 8 Alkhateeb A, Stetler GL, Old W, Talbert J, Uhlhorn C, Taylor M, Fox A, Miller C, Dills DG, Ridgway EC, Bennett DC, Fain PR, Spritz RA: Mapping of an autoimmunity susceptibility locus (*AISI*) to chromosome 1p31.3–p32.2. *Hum Mol Genet* 2002;11:661–667.
- 9 Mehta NR, Shah KC, Theodore C, Vyas VP, Patel AB: Epidemiological study of vitiligo in Surat area. *Indian J Med Res* 1973;61:145–154.
- 10 Carnevale A, Zavala C, Castillo VD, Maldonado RR, Tamayo L: Analisis genetico de 127 families con vitiligo. *Rev Invest Clin* 1980;32:37–41.
- 11 Hafez M, Sharaf L, Abd El-Nabi SM: The genetics of vitiligo. *Acta Derm Venereol* 1983;63: 249–251.

- 12 Das SK, Majumder PP, Majumder TK, Haldar B: Studies on vitiligo. II. Familial aggregation and genetics. *Genet Epidemiol* 1985;2:255–262.
- 13 Majumder PP, Das SK, Li CC: A genetical model for vitiligo. *Am J Hum Genet* 1988;43:119–125.
- 14 Bhatia PS, Mohan L, Pandey ON, Singh KK, Arora SK, Mukhija RD: Genetic nature of vitiligo. *J Dermatol Sci* 1992;4:180–184.
- 15 Majumder PP, Nordlund JJ, Nath SK: Pattern of familial aggregation of vitiligo. *Arch Dermatol* 1993;129:994–998.
- 16 Nath SK, Majumder PP, Nordlund JJ: Genetic epidemiology of vitiligo: multilocus recessivity cross-validated. *Am J Hum Genet* 1994;55:981–990.
- 17 Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA: Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their relatives. *Pigment Cell Res* 2003;16:208–214.
- 18 Sun X, Xu A, Wei X, Ouyang J, Lu L, Chen M, Zhang D: Genetic epidemiology of vitiligo: a study of 815 probands and their families from south China. *Int J Dermatol* 2006;45:1176–1181.
- 19 Laberge G, Mailloux CM, Gowan K, Holland P, Bennett DC, Fain PR, Spritz RA: Early disease onset and increased risk of other autoimmune diseases in familial generalized vitiligo. *Pigment Cell Res* 2005;18:300–305.
- 20 Harsoulis P, Kanakoudi-Tsakalidis F, Vyzantiadis A, Minas A, Cassimos C: Autoimmunity and vitiligo. *Arch Dermatol* 1978;114:1554.
- 21 Ongenaes K, Van Geel N, Naeyaert J-M: Evidence for an autoimmune pathogenesis of vitiligo. *Pigment Cell Res* 2003;16:90–100.
- 22 Le Poole CI, Wankowicz-Kalinska A, van den Wijngaard RMJGJ, Nickoloff BJ, Das DK: Autoimmune aspects of depigmentation in vitiligo. *J Invest Dermatol Symp Proc* 2004;9:68–72.
- 23 Kemp EH, Gavalas NG, Gawkrödger DJ, Weetman AP: Autoantibody responses to melanocytes in the depigmenting skin disease vitiligo. *Autoimmun Rev* 2007;6:138–142.
- 24 Ogg GS, Rod Dunbar P, Romero P, Chen JL, Cerundolo V: High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. *J Exp Med* 1998;188:1203–1208.
- 25 Gross A, Tapia FJ, Mosca W, Perez RM, Briceno L, Henriquez JJ, Convit J: Mononuclear cell subpopulations and infiltrating lymphocytes in erythema dyschromicum perstans and vitiligo. *Histol Histopathol* 1987;2:277–283.
- 26 Badri AM, Todd PM, Garioch JJ, Gudgeon JE, Stewart DG, Goudie RB: An immunohistological study of cutaneous lymphocytes in vitiligo. *J Pathol* 1993;170:149–155.
- 27 Le Poole IC, van den Wijngaard RM, Westerhof W, Das PK: Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am J Pathol* 1996;148: 1219–1228.
- 28 Cunliffe W, Hall R, Newell J, Stevenson CJ: Vitiligo, thyroid disease and autoimmunity. *Br J Dermatol* 1968;80:135–139.
- 29 Schallreuter KW, Lemke R, Brandt O, Schwartz R, Westhofen M, Montz R, Berger J: Vitiligo and other diseases: coexistence or true association? Hamburg study on 321 patients. *Dermatology* 1994;188:269–275.
- 30 Dawber R: Integumentary associations of pernicious anemia. *Br J Dermatol* 1969;82:221–222.
- 31 Grunnet I, Howitz J: Vitiligo and pernicious anemia. *Arch Dermatol* 1979;101:82–85.
- 32 Zelissen PMJ, Bast EJEG, Croughs RJM: Associated autoimmunity in Addison's disease. *J Autoimmun* 1995;8:121–130.
- 33 Sharma VK, Dawn G, Kumar B: Profile of alopecia areata in Northern India. *Int J Dermatol* 1996;35:22–27.
- 34 Sharma VK, Kumar V, Dawn G: A clinical study of childhood alopecia areata in Chandigarh, India. *Pediatr Dermatol* 1996;13:372–377.
- 35 Handa S, Kaur I: Vitiligo: clinical findings in 1,436 patients. *J Dermatol* 1999;26:653–657.
- 36 Handa S, Dogra S: Epidemiology of childhood vitiligo: a study of 625 patients from north India. *Pediatr Dermatol* 2003;20:207–210.
- 37 Onunu AN, Kubeyinje EP: Vitiligo in the Nigerian African: a study of 351 patients in Benin City, Nigeria. *Int J Dermatol* 2003;42:800–802.
- 38 Le Poole IC, Sarangarajan R, Zhao Y, Stennett LS, Brown TL, Sheth P, Miki T, Boissy RE: 'VIT1', a novel gene associated with vitiligo. *Pigment Cell Res* 2001;14:475–484.

- 39 Kingo K, Philips MA, Aunin E, Luuk H, Karelson M, Ratsep R, Silm H, Vasar E, Koks S: MYG1, novel melanocyte related gene, has elevated expression in vitiligo. *J Dermatol Sci* 2006;44: 119–122.
- 40 Foley LM, Lowe NJ, Misheloff E, Tiwari JL: Association of HLA-DR4 with vitiligo. *J Am Acad Dermatol* 1983;8:39–40.
- 41 Finco O, Cuccia M, Mantinetti M, Ruberto G, Orecchia G, Rabbiosi G: Age of onset in vitiligo: relationship with HLA supratypes. *Clin Genet* 1991;39:448–454.
- 42 Orecchia G, Perfetti L, Malagoli P, Borghini F, Kipervarg Y: Vitiligo is associated with a significant increase in HLA-A30, Cw6 and Dqw3 and a decrease in C4AQ0 in northern Italian patients. *Dermatology* 1992;185:123–127.
- 43 Ando I, Chi HI, Nakagawa H, Otsuka F: Difference in clinical features and HLA antigens between familial and non-familial vitiligo of non-segmental type. *Br J Dermatol* 1993;129:408–410.
- 44 Schallreuter KU, Levenig C, Kuhl P, Loliger C, Hohl-Tehari M, Berger J: Histocompatibility antigens in vitiligo: Hamburg study on 102 patients from northern Germany. *Dermatology* 1993;187:186–192.
- 45 al-Fouzan A, al-Arbash M, Fouad F, Kaaba SA, Mousa MA, al-Harbi SA: Study of HLA class I/II and T lymphocyte subsets in Kuwaiti vitiligo patients. *Eur J Immunogenet* 1995;22:209–213.
- 46 Zamani M, Spaepen M, Sghar SS, Huang C, Westerhof W, Nieuweboer-Krobotova L, Cassiman JJ: Linkage and association of HLA class II genes with vitiligo in a Dutch population. *Br J Dermatol* 2001;145:90–94.
- 47 Arcos-Burgos M, Parodi E, Salgar M, Bedoya E, Builes JJ, Jaramillo D, Ceballos G, Uribe A, Rivera N, Rivera D, Fonseca I, Camargo M, Palacio LG: Vitiligo: complex segregation and linkage disequilibrium analyses with respect to microsatellite loci spanning the HLA. *Hum Genet* 2002;110:334–342.
- 48 Tastan HB, Akar A, Orkunoglu FE, Arca E, Inal A: Association of HLA class I antigens and HLA class II alleles with vitiligo in a Turkish population. *Pigment Cell Res* 2004;17:181–184.
- 49 Xia Q, Zhou WM, Liang YH, Ge HS, Liu HS, Wang JY, Gao M, Yang S, Zhang XJ: MHC haplotypic association in Chinese Han patients with vitiligo. *J Eur Acad Dermatol Venereol* 2006;20: 941–946.
- 50 Liu JB, Li M, Chen H, Zhong SQ, Yang S, Du WD, Hao JH, Zhang TS, Zhang XJ, Zeegers M: Association of vitiligo with HLA-A2: a meta-analysis. *J Eur Acad Dermatol Venereol* 2007;21:205–213.
- 51 Fain PR, Babu SR, Bennett DC, Spritz RA: HLA class II haplotype DRB1*04-DQB1*0301 contributes to risk of familial generalized vitiligo and early disease onset. *Pigment Cell Res* 2006;19: 51–57.
- 52 Casp CB, She JX, McCormack WT: Genes of the LMP/TAP cluster are associated with the human autoimmune disease vitiligo. *Genes Immun* 2003;4:492–499.
- 53 Chen JJ, Huang W, Gui JP, Yang S, Zhou FS, Xiong OG, Wu HB, Cui Y, Gao M, Li W, Li JZ, Yan KL, Yuan WT, Xu SJ, Liu JJ, Zhang XJ: A novel linkage to generalized vitiligo on 4q13–q21 identified in a genomewide linkage analysis of Chinese families. *Am J Hum Genet* 2005;76: 1057–1065.
- 54 De la Fuente-Fernandez R: Mutations in GTP-cyclohydrolase I gene and vitiligo. *Lancet* 1997; 350:640.
- 55 Bandyopadhyay D, Lawrence E, Majumder PP, Ferrell RE: Vitiligo is not caused by mutations in GTP-cyclohydrolase I gene. *Clin Exper Dermatol* 2000;25:152–153.
- 56 Casp CB, She JX, McCormack WT: Genetic association of the catalase gene (CAT) with vitiligo susceptibility. *Pigment Cell Res* 2002;15:62–66.
- 57 Gavalas NG, Akhtar S, Gawkrödger DJ, Watson PF, Weetman AP, Kemp EH: Analysis of allelic variants in the catalase gene in patients with the skin depigmenting disorder vitiligo. *Biochem Biophys Res Commun* 2006;14;345:1586–1591.
- 58 Park HH, Ha E, Uhm YK, Jin SY, Kim YJ, Chung JH, Lee MH: Association study between catalase gene polymorphisms and the susceptibility to vitiligo in Korean population. *Exp Dermatol* 2006;15:377–380.
- 59 Jin SY, Park HH, Li GZ, Lee HJ, Hong MS, Hong SJ, Park HK, Chung JH, Lee MH: Association of angiotensin converting enzyme gene I/D polymorphism of vitiligo in Korean population. *Pigment Cell Res* 2004;17:84–86.

- 60 Akhtar S, Gavalas NG, Gawkrödger DJ, Watson PF, Weetman AP, Kemp EH: An insertion/deletion polymorphism in the gene encoding angiotensin converting enzyme is not associated with generalised vitiligo in an English population. *Arch Dermatol Res* 2005;297:94–98.
- 61 Jin SY, Park HH, Li GZ, Lee HJ, Hong MS, Park HJ, Park HK, Seo JC, Yim SV, Chung JH, Lee MH: Association of estrogen receptor 1 intron 1 C/T polymorphism in Korean vitiligo patients. *J Dermatol Sci* 2004;35:181–186.
- 62 Tursen U, Kaya TI, Erdal ME, Derici E, Gunduz O, Ikizoglu G: Association between catechol-O-methyltransferase polymorphism and vitiligo. *Arch Dermatol Res* 2002;294:143–146.
- 63 Birlea S, Birlea M, Cimponeriu D, Apostol P, Cosgarea R, Gavrilă L, Tigan S, Costin G, Das P: Autoimmune diseases and vitamin D receptor Apa-I polymorphism are associated with vitiligo in a small inbred Romanian community. *Acta Derm Venereol* 2006;86:209–214.
- 64 Blomhoff A, Kemp EH, Gawkrödger DJ, Weetman AP, Husebye ES, Akselsen HE, Lie BA, Undlien DE: CTLA4 polymorphisms are associated with vitiligo, in patients with concomitant autoimmune diseases. *Pigment Cell Res* 2005;18:55–58.
- 65 Canton I, Akhtar S, Gavalas NG, Gawkrödger DJ, Blomhoff A, Watson PF, Weetman AP, Kemp EH: A single-nucleotide polymorphism in the gene encoding lymphoid protein tyrosine phosphatase (PTPN22) confers susceptibility to generalised vitiligo. *Genes Immun* 2005;6:584–587.
- 66 Onay H, Pehlivan M, Alper S, Ozkinay F, Pehlivan S: Might there be a link between mannose binding lectin and vitiligo? *Eur J Dermatol* 2007;17:146–148.
- 67 Brand O, Gough S, Heward J: HLA, CTLA-4, and PTPN22: the shared genetic master-key to autoimmunity? *Expert Rev Mol Med* 2005;7:1–15.
- 68 Tripathi RK, Flanders DJ, Young TL, Oetting WS, Ramaiah A, King RA, Boissy RE, Nordlund JJ: Microphthalmia-associated transcription factor (MITF) locus lacks linkage to human vitiligo or osteopetrosis: an evaluation. *Pigment Cell Res* 1999;12:187–192.
- 69 Nath SK, Kelly JA, Namjou B, Lam T, Bruner GR, Scofield RH, Aston CE, Harley JB: Evidence for a susceptibility gene, *SLEVI*, on chromosome 17p13 in families with vitiligo-related systemic lupus erythematosus. *Am J Hum Genet* 2001;69:1401–1406.
- 70 Alkhateeb A, Fain PR, Spritz RA: Candidate functional promoter variant in the *FOXD3* melanoblast developmental regulator gene in a family with autosomal dominant vitiligo. *J Invest Dermatol* 2005;125:388–391.
- 71 Fain PR, Gowan K, LaBerge GS, Alkhateeb A, Stetler GL, Talbert J, Bennett DC, Spritz RA: A genomewide screen for generalized vitiligo: confirmation of *AISI* on chromosome 1p31 and evidence for additional susceptibility loci. *Am J Hum Genet* 2003;72:1560–1564.
- 72 Spritz RA, Gowan K, Bennett DC, Fain PR: Novel vitiligo susceptibility loci on chromosomes 7 (*AIS2*) and 8 (*AIS3*), confirmation of *SLEVI* on chromosome 17, and their roles in an autoimmune diathesis. *Am J Hum Genet* 2004;74:188–191.
- 73 Ying J, Mailloux CM, Gowan K, Riccardi SL, LaBerge G, Bennett DC, Fain PR, Spritz RA: *NALP1* in vitiligo-associated multiple autoimmune disease. *N Engl J Med* 2007;356:1216–1225.
- 74 Johansson CM, Zunc R, Garcia MA, Scherbarth HR, Tate GA, Pairs S, Navarro SM, Perandones CE, Gamron S, Alvarellos A, Graf CE, Manni J, Berbotto GA, Palatnik SA, Catoggio LJ, Battagliotti CG, Sebastiani GD, Migliaresi S, Galeazzi M, Pons-Estel BA, Alarcon-Riquelme ME; Collaborative Group on the Genetics of SLE; Argentine Collaborative Group: Chromosome 17p12–q11 harbors susceptibility loci for systemic lupus erythematosus. *Hum Genet* 2004;115: 230–238.
- 75 Jin Y, Birlea SA, Fain PR, Spritz RA: Genetic variations in *NALP1* are associated with generalized vitiligo in a Romanian population. *J Invest Dermatol* 2007;127:2558–2562.

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Scleroderma

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Abstract

The prototypic autoimmune diseases involving skin (lupus, dermatomyositis) typically result in epithelial injury and autoantibodies to characteristic cellular antigens. Disease-specific autoantibodies are also found in scleroderma, but scleroderma is different from other cutaneous autoimmune diseases because epithelial injury does not occur. Multiple factors and combinations of factors (immune system, vascular and extracellular matrix abnormalities) are the most likely triggers in an individual with a genetic predisposition to scleroderma. These lead to increased synthesis of normal collagen in skin, lungs and gut in the systemic form of scleroderma, systemic sclerosis. The hypotheses for the pathophysiology of scleroderma are diverse and include abnormal immunologic processes such as cytokine and chemokine dysregulation, abnormal T cell signaling, B cell dysfunction, injury due to autoantibodies to endothelial cells, persistent wound healing condition due to dysregulation of matrix homeostasis, abnormalities in the fibrinolytic system, polymorphisms in critical molecules of the immune system and matrix homeostasis, and microchimerism due to fetal/maternal placental exchange of HLA-compatible cells. Systemic sclerosis/scleroderma is chronic and progressive. To date, no definitive therapy is effective for any of the scleroderma variants, although agents for the vascular dysfunction have some utility. Hematopoietic bone marrow or stem cell transplantation before significant tissue fibrosis has occurred may be the most effective treatment.

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Introduction to Scleroderma

Scleroderma as an Autoimmune Disease

Scleroderma (systemic sclerosis) is a debilitating chronic autoimmune disease of unknown etiology. It typically has an insidious onset and can lead to extensive progressive fibrosis of skin and viscera due to excessive production of normal collagen. The classic autoimmune features consist of autoantibodies to nuclear, nucleolar and cytoplasmic antigens, and to endothelial cells. The

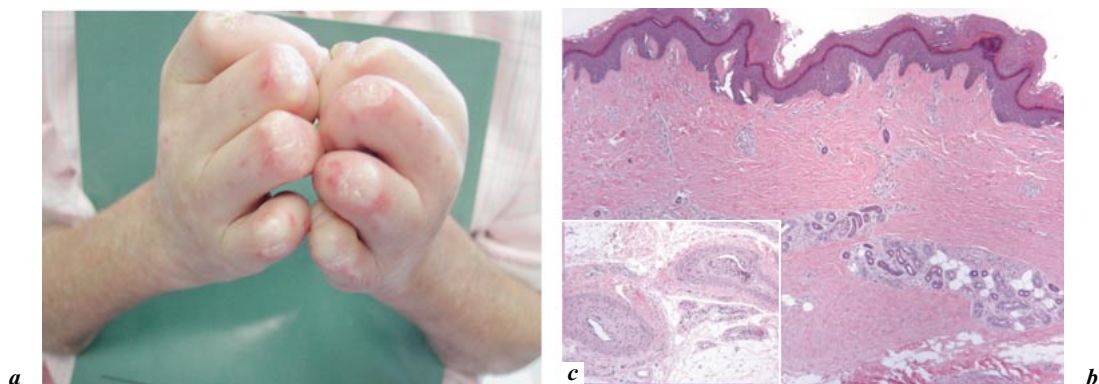


Fig. 1. *a* Typical clinical appearance of diffuse scleroderma (courtesy of Dr. Mamood Pazirandeh). *b, c* Histology of skin fibrosis and prominent proliferative and obliterative vascular change.

autoantibodies are important for the classification of subsets of scleroderma and in some instances, they may be pathogenic.

Scleroderma is unlike other autoimmune diseases that involve skin because there appears to be no direct injury to epithelia as in lupus erythematosus, dermatomyositis or alopecia areata. Rather, abnormal upregulation of collagen synthesis and progressive vascular occlusion are the hallmarks of disease, accompanied by alterations in cytokines, chemokines and growth factors, and production of disease-specific autoantibodies. Except for the endothelial cell autoantibodies, the major scleroderma autoantibodies do not appear to cause direct injury to tissue, and no direct injury by T cells occurs. Rather, the tissue cytokine and chemokine environments, vascular injury and imbalance in the fibrinolytic system enhance collagen deposition and vascular occlusion. The result is skin, lung, gut fibrosis and renal vessel fibrosis in systemic forms of scleroderma. The clinical features can include sequelae of vascular compromise and ischemia such as Raynaud's phenomenon and digital ulcers.

Severity and morbidity of disease depend on the degree of internal involvement and extent of cutaneous fibrosis. The typical clinical appearance of diffuse scleroderma is shown in figure 1a and the histology of skin fibrosis and prominent proliferative and obliterative vascular change in figures 1b and c. There are two other forms of scleroderma which cannot be distinguished from each other or from diffuse scleroderma histologically, but are quite distinct clinically. They are limited scleroderma (CREST) and linear scleroderma/morphea. For this review, we will concentrate on the immunopathophysiology and newest concepts of disease related to human systemic sclerosis/scleroderma, which has been best characterized and has the worst prognosis.

Table 1. Factors that contribute to pathogenesis in scleroderma

Immune dysregulation	–growth factor, cytokine and chemokine environments –T cell signaling –B cell abnormalities –autoantibodies
Extracellular matrix dysregulation	–differentiation to myofibroblasts –fibroblast signaling pathways –altered fibroblast apoptosis –altered metalloproteinases
Vascular injury	–anti-endothelial cell antibodies –cytomegalovirus infection
Genetic factors	–HLA associations –polymorphisms
Microchimerism of fetal/maternal cells	
Environmental triggers	–silica –solvents

Incidence and Demographics of Scleroderma

New data on the incidence in the US in black and Caucasian adults estimate scleroderma at approximately 276 cases per million adults (95% confidence interval 245–310) [1]. Women and blacks are more likely to have scleroderma, and blacks are twice as likely to have diffuse systemic disease as whites. Median survival in diffuse disease is 11 years. Male sex and older age at diagnosis are negative factors for survival [1].

What Causes Scleroderma?

There are many hypotheses for the triggering events leading to scleroderma, but none completely address the spectrum of clinical and molecular findings. Some of these hypotheses are listed in table 1 and include abnormal immunologic processes such as cytokine and chemokine dysregulation, abnormal T cell signaling, B cell dysfunction, injury due to autoantibodies to endothelial cells, persistent wound healing condition due to dysregulation of matrix homeostasis, abnormalities in the fibrinolytic system, and microchimerism due to fetal/maternal placental exchange of HLA-compatible cells. Genetic studies on scleroderma in the Oklahoma Choctaw Indians and in well-defined ethnic populations of individuals with scleroderma (American blacks, Japanese), as well as linked HLA haplotypes and autoantibody phenotypes suggest a genetic predisposition to scleroderma. There are also gene polymorphisms involving molecules of the immune and cellular regulatory system that are associated with scleroderma in

subgroups of individuals. These all point to the critical and central role that genetics may play in scleroderma. Lastly, there are environmental triggers such as silica, toxic oils and solvents that have been implicated in scleroderma-like disease and are reviewed by Nietert and Silver [2].

Therefore, multiple factors and combinations of factors (immune system, vascular and extracellular matrix abnormalities) are the most likely triggers in an individual with a genetic predisposition to scleroderma. The variety of suspects suggests a complex multigenic disease, possibly with many overlapping pathways to fibrosis.

In vitro Studies

Information on molecular events in autoimmune fibrosing disease has come mainly from in vitro studies of fibroblasts isolated from skin of individuals with scleroderma. Mapping out the molecular pathways leading to the excessive collagen production by scleroderma fibroblasts (TGF- β , Smad signaling pathway) has increased our understanding of the fibroblast component of disease. A caveat for the fibroblast studies is that gene array profiles from cultured human scleroderma skin fibroblasts are incomplete compared with freshly isolated scleroderma skin cells [3].

Animal Models

Studies on several useful animal models reviewed separately have broadened our understanding of scleroderma [for review, see 4–6], including murine bleomycin-induced fibrosis [7, 8], murine sclerodermatous graft-versus-host disease [9–16], the UC Davis (UCD 200) chicken model [17, 18], the tight skin (TSK1) mouse [19] and several transgenic and knockout mouse models [6]. Backcrosses with mutant strains, conditional reporter transgenic lines for the TSK1 line and TGF- β receptor transgenic mice provide an exciting opportunity to dissect out the key events and pathways in scleroderma [20, 21]. Again, no single one of these demonstrates the entire spectrum of clinical findings in scleroderma patients. Rather, each one displays subsets of features, much like the clinical spectrum of scleroderma. The presence of multiple animal models, each representing a different process leading to the end point of tissue fibrosis, also suggests multiple possible etiologies of autoimmune fibrosing disease.

New Developments in Scleroderma Research

There are exciting new advances in other areas that relate to scleroderma. For instance, the concept of fibroblast patterning (different classes of cutaneous fibroblasts based on gene array analysis of skin fibroblasts from different regions of the body) [22] helps to explain the localization of early scleroderma to hands, feet and face, which all contain a distal-type fibroblast.

The new developments in scleroderma research occur in these diverse areas: immune dysregulation including chronic B cell activation, matrix dysregulation, molecular characterization of autoantibodies, microvascular dysfunction, genetic polymorphisms, and microchimerism (table 1). Linking the new concepts and findings in a coherent hypothesis for disease remains the challenge in our understanding of scleroderma [6, 23–31].

Therapy for Scleroderma

Evaluation of therapy is difficult because the clinical disease is heterogeneous and can wax and wane. Global immunosuppression with corticosteroids or other immunosuppressants is not effective and leads to unwanted side effects with long-term use. D-penicillamine, minocycline, recombinant relaxin, tamoxifen, extracorporeal photopheresis, interferon (IFN)- α and IFN- γ all failed as effective therapies in clinical trials (<http://www.sclero.org/medical/research/clinical-trials/a-to-z.html>). A few agents are useful for the vascular dysfunction in selected patients. Current effective therapies for scleroderma are as follows [32]:

- Renal crisis: angiotensin-converting enzyme (ACE) inhibitors alter the rennin-angiotensin axis
- Pulmonary hypertension:
 - epoprostenol, treprostenil and iloprost supply prostaglandins not provided by the pulmonary arterial vasculature
 - sildanefil citrate (Viagra) elevates nitric oxide, a vasodilator
 - bosentan inhibits endothelin which is abnormally increased in pulmonary hypertension
- Pulmonary fibrosis: cyclophosphamide, an alkylating cytotoxic agent, affects cell replication but is also an immunosuppressant
- Raynaud's disease: calcium channel blocking agents affect movement of calcium in endothelium and act as vasodilators
- Digital ulcers: bosentan prevents new ulcers
- Skin fibrosis: methotrexate inhibits immune cell activation and inflammation

Examples of clinical trials using therapeutic agents for vascular disease are:

- Bosentan in a variety of clinical trials for digital ulcers, pulmonary fibrosis, pulmonary hypertension, Raynaud's disease and skin fibrosis (multiple centers)
- Quinipril (ACE inhibitor) for systemic sclerosis (Gwynedd Hospital, North Wales)

In addition, several different novel therapies (such as monoclonal antibodies to cytokines and cytokine receptors, immunosuppressive regimens and phototherapy) that alter cytokine and chemokine environments are reported in small patient cohorts. The benefits must always be weighed against the risks of

altering homeostasis in the complex networks of the immune system [33]. Examples of trials using these immunomodulatory agents are summarized below (<http://www.sclero.org/medical/research/clinical-trials/a-to-z.html>):

- Recombinant human anti-TGF- β antibody (CAT 192) is no better than placebo (Royal Free Hospital, England)
- Infliximab (TNF- α inhibitor) stabilizes systemic disease parameters but has serious side effects including pulmonary hypertension, digital ulcers and Raynaud's disease (Royal Free Hospital, England)
- Intravenous immunoglobulin for immunosuppression (multiple centers)
- PVAC, a therapy derived from delipidated, deglycolipidated *Mycobacterium vaccae* for diffuse scleroderma (Stanford University, University of San Diego)
- Thalidomide for diffuse scleroderma (New York University)
- PUVA UVA-1 for diffuse and limited scleroderma and morphea (multiple centers)

The variety of agents in trials points to the refractory nature of scleroderma to treatment. At present, scleroderma is typically a chronic, debilitating, relentlessly progressive disease with few if any definitive therapies. Bone marrow or stem cell transplantation may be the only definite cure for scleroderma, and this option is available in experimental trials. It is effective in early but not late disease when significant tissue injury is already present [34]. Some examples of clinical trials using transplantation are summarized below (<http://www.sclero.org/medical/research/clinical-trials/a-to-z.html>):

- SCOT clinical trial comparing stem cell transplantation or high-dose cyclophosphamide (Johns Hopkins University)
- Allogeneic hematopoietic stem cell transplantation of sibling donor stem cells into scleroderma individuals previously treated with cyclophosphamide, fludarabine and Campath 1H for immunosuppression (Northwestern University)
- ASTIS study: high-dose immunoablation and hematopoietic stem cell transplantation versus monthly intravenous pulse therapy cyclophosphamide [European Group for Blood and Marrow Transplantation (EBMT)/European League Against Rheumatism (EULAR) Scleroderma Study Group]

How Do We Know When Therapy Works?

The groundwork for evaluation of potential therapies for scleroderma was laid by Metzger and Steen in the 1970s. They developed an extensive longitudinal database with demographics, clinical information and outcomes for scleroderma patients treated at the University of Pittsburgh. Multicenter trials developed in the 1980s and 1990s provided the power of additional patient

numbers and standardized therapy protocols. These important advances led to the 6th and 7th Outcome Measures in Rheumatology Clinical Trials (OMER-ACT), which provided guidelines for different outcome measurements for clinical trials in scleroderma [35, 36]; however, at present there is no consensus on how to evaluate disease activity or prognostic criteria in general clinical practice.

New Concepts in Scleroderma Pathophysiology

Immune Dysregulation in Scleroderma

Historically, established scleroderma was thought to be predominantly a T cell process with increased activated T cells in the blood and tissue. These bear restricted T cell receptor repertoires and are associated with Th2-like cytokine profiles based on RT-PCR of cells from blood and tissue and by ELISA [37–39].

Cytokines

TGF- β , which is the prototypic profibrotic cytokine, increases collagen synthesis by fibroblasts and downregulates extracellular matrix degradation. TGF- β is upregulated in scleroderma, driving collagen type I synthesis via connective tissue growth factor (CTGF), a downstream regulator of collagen synthesis [40]. Other profibrotic cytokines that may play a role in scleroderma are IL-4, IL-6 and IL-13 [for review, see 39, 41]. Cytokine-directed therapy is proposed as a logical extension of the extensive knowledge base on cytokines and scleroderma [42] but has not been effective, to date.

Chemokines

Individuals with scleroderma have increased serum monocyte chemoattractant factor-1 (MCP-1) [43], cutaneous T cell-attracting chemokine (CTAC) [44], regulated on activation normal T cell expressed and secreted (RANTES) and IP-10 by a variety of methods [for review, see 39, 45]. Chemokine receptors such as the MCP-1 receptor are also upregulated [46]. The combination of chemokines for both T cells and macrophages is consistent with the presence of both types of immune cells in scleroderma skin.

Transcriptional profiles for global gene expression of peripheral blood cells have revealed some novel immune markers for scleroderma. Of note, a large number of IFN- γ -inducible and vasculotrophic genes are upregulated compared to normal controls. Several signaling pathways are also upregulated compared to controls: insulin growth factor-1 and insulin, epidermal growth factor, insulin, antiapoptosis factors, and platelet-derived growth factor, among others [47]. Evaluation of these pathways in vivo and in vitro is ongoing.

B Cell Abnormalities and Scleroderma

The role of B cells in the normal immune response is to make immunoglobulin and participate in immune regulation. In the past, scleroderma was thought to be mainly a T cell disorder. However, it has been shown recently that individuals with systemic sclerosis have expanded naïve B cells and decreased but activated memory T cells compared to normal individuals. Several abnormalities in the B cell immune axis may explain this phenomenon.

- Individuals with diffuse scleroderma overexpress CD19, a marker for B cell regulation, by approximately 20%, which may produce chronic B cell activation and lead to autoantibody production [48].
- Polymorphisms of the promoter for CD19 may explain in part the chronic B cell activation and increased production of IL-6, a profibrotic cytokine elevated in scleroderma. Abnormal B cell function may also influence T cell activation and function, also leading to a tissue environment that enhances fibrosis [49, 50].
- Serum levels of B cell-activating factor (BAFF, a member of the TNF- α family of ligands and a survival factor for B cells) and BAFF receptor on B cells are elevated in diffuse scleroderma. Increased serum BAFF correlates with the extent and exacerbation of disease. Similarly, elevated mRNA for BAFF is seen in early diffuse scleroderma but not in normal skin. B cells isolated from individuals with diffuse scleroderma have an enhanced ability to produce IL-6 and immunoglobulin when exposed to BAFF [51].

The abnormalities in multiple cytokines, chemokines and growth factors in scleroderma suggest a complex regulatory immune network that involves multiple pathways and molecules. Interfering with one molecule may inadvertently change the balance in another. The challenge in scleroderma therapy is to target the critical pathways in fibrosing disease without disruption of the normal ones and subsequent adverse effects.

Major Autoantibodies in Scleroderma

Individuals with scleroderma can be grouped into several nonoverlapping categories based on the presence of characteristic disease-specific autoantibodies in their sera: anti-centromere (limited cutaneous disease and pulmonary vascular disease), anti-Scl-70/topoisomerase I (diffuse systemic disease and diffuse pulmonary fibrosis), anti-fibrillarin/anti-U3 nucleolar antigens (diffuse systemic disease) and anti-RNA polymerase I–III (diffuse systemic disease) [for review, see 52–56]. The anti-fibrillarin/U3 and anti-RNA polymerase antibodies are less commonly found in scleroderma sera. Other autoantibodies historically described in small groups of scleroderma patients are Pm/Scl (limited cutaneous disease) and anti-Th/To (limited cutaneous disease but likely a marker for pulmonary hypertension).

Less Common Autoantibodies in Scleroderma

There are rare autoantibodies in scleroderma sera that are also found in other autoimmune diseases and are not useful for clinical diagnosis or correlation with disease activity: anti-Ku which is also found in lupus erythematosus [52] and anti-U1 which is found in mixed connective tissue disease [57]. Anti-Ro autoantibodies are occasionally found in scleroderma patients and are a marker for sicca syndrome. A list of other rare autoantibodies reported in the last 5 years in scleroderma is given in table 2. Some are associated with subgroups of individuals with scleroderma, but none have yet been shown to be directly pathogenic. They may simply reflect immune system dysfunction in autoimmunity. They are worth noting because the autoantibodies in scleroderma and other autoimmune diseases have been used as tools to identify, characterize and in some cases, purify important molecules of the cell. The autoantibodies of scleroderma have been the basis for important advances in molecular and cell biology because they target well-conserved critical molecules essential to the cell.

A few autoantibodies in scleroderma may be pathogenic [30]. They are directed to endothelial cells, fibroblasts, fibrillin, matrix metalloproteinases and platelet-derived growth factor (PDGF).

Anti-Endothelial Cell Antibodies

Anti-endothelial cell antibodies (AECA) are a newly described group of antibodies that may play a major role in pathogenesis of scleroderma. They are found mainly in limited scleroderma and bind to the ubiquitous centromeric nuclear protein CENP-B [58]. AECA have been identified *in vivo* in serum and directly in fibrotic lung tissue in scleroderma [59].

Anti-Fibroblast Antibodies

Anti-fibroblast antibodies have also been described in scleroderma sera and may be pathogenic. They bind to the surface of human and rodent fibroblasts. Anti-topoisomerase antibodies also have some anti-fibroblast binding properties. The binding of purified topoisomerase antigen to fibroblasts in culture and subsequent binding of anti-topoisomerase antibodies causes activation and adhesion of cocultured macrophages. This suggests a pathogenic role for the anti-topoisomerase antibodies in amplification of the fibrogenic cascade [60].

Anti-PDGF and PDGF Receptor Antibodies

PDGF stimulates production of reactive oxygen species, which can damage vascular endothelium [61, 62].

Table 2. Rare new autoantibodies in scleroderma

Antigen	Function	Association	Reference
<i>ECM molecules</i>			
Fibrillin-1	Component of ECM microfibrils and regulator of TGF- β by sequestration in ECM; duplicated in TSK1 mouse	Choctaw Indians with scleroderma	[100, 101]
MMP-1 and MMP-3	Enzymes that degrade ECM	Rheumatoid arthritis	[102–104]
PHET	Ectopic expression in scleroderma fibroblasts	Lung disease	[105]
<i>Nuclear and nucleolar regulatory molecules</i>			
ReqQ3 RNA/DNA helicase (WRN)	Nuclear enzyme mutated in Werner syndrome		[106]
U1 RNP	Component of the small nuclear RNP involved in mRNA splicing	Esophageal and lung disease	[57, 107, 108]
U3 RNP			
Fibrillarin			
B23	Nucleolar phosphoprotein	Pulmonary hypertension	[109]
HSP47	Collagen-specific molecular chaperone with role in collagen homeostasis in fibroblasts		[110]
Ufd2 complex	E4 polyubiquitylating enzyme, regulates chromosome and separation in mitosis condensation		[111]
<i>Vascular antigens</i>			
Phosphotidyl serine prothrombin complex	β 2 glycoprotein in resting endothelial cells	Thrombo-embolism, peripheral ischemia and pulmonary hypertension	[54, 112]
Cardiolipin			

ECM = Extracellular matrix; MMP = matrix metalloproteinase; PHET = protein highly expressed in testis; RNP = ribonucleoprotein; HSP = heat shock protein.

Autoantigens unique to diffuse scleroderma (that is, topoisomerase I) have metal binding sites, and are susceptible to metal ion-associated oxidation reactions and cleavage. These data support the vascular injury/reperfusion hypothesis for scleroderma [63, 64]. The search for an abnormality in metal ion status in scleroderma has not yielded any candidate metal ions, however.

Innate Immunity

Very little is known about the role of innate immunity in scleroderma.

Extracellular Matrix Dysregulation

There is an extensive literature on fibroblast biology in scleroderma [for review, see 41, 65–67]. Fibroblasts are collected from scleroderma skin or lung in individuals with established disease and cultured for 6–8 passages for in vitro studies. The concepts distilled from these experiments have been important in understanding molecular pathways that could lead to the abnormal collagen synthesis of scleroderma. However, recent data from gene array studies comparing cultured scleroderma fibroblasts and freshly isolated scleroderma skin cells have shown that the cultured fibroblasts provide an incomplete picture of in vivo cell transcription profiles [3]. With that caveat in mind, several critical pathways in fibroblast biology and collagen regulation have been identified.

There are several interesting populations of fibroblast-related cells that may be involved in abnormal skin fibrosis in scleroderma.

- Fibrocytes: the peripheral blood contains a population of CD34+ collagen-positive cells that may function in wound healing and may have a role in fibrosing disorders such as scleroderma [68]. Fibrocytes may be a source of myofibroblasts.
- The myofibroblast, proposed to originate from fibroblasts, pericytes and possibly monocytes, expresses α smooth muscle actin, Thy-1 and EDA-fibronectin [69]. TGF- β induces the differentiation of myofibroblasts; increased numbers of myofibroblasts are seen in scleroderma but not in control skin [66, 68]. TGF- β also inhibits the apoptosis of fibroblasts and myofibroblast lineage cells, suggested as a mechanism for the abnormal scleroderma phenotype [27, 70, 71].

Smads are intracellular signal-transducing molecules and activators of the fibroblast collagen transcription regulating pathway [72]. TGF- β interacts with the transmembrane type I activin-like receptor kinase (ALK5) TGF- β receptor. The receptor is linked to Smad 2/3 proteins which activate the pathway. Smad 7 inhibits the pathway.

- Protein and mRNA levels of Smad 3 but not Smad 4 or Smad 7 are increased in scleroderma fibroblasts compared to matched normal controls, suggesting

that the Smads play a critical role in maintenance of the scleroderma fibroblast phenotype [73].

- Others have shown deficient Smad 7 in scleroderma fibroblasts [74].

Endothelin-1, a vasoconstrictor peptide derived from endothelium and also synthesized by fibroblasts, is also important for extracellular matrix homeostasis via endothelin receptors. Endothelin-1 in vitro promotes fibroblast synthesis of collagen, inhibits metalloproteinase synthesis, induces myofibroblast differentiation and upregulates ICAM-1 which could affect immune cell-fibroblast adhesion [75–77]. TGF- β can induce endothelin synthesis by fibroblasts via the JNK/AP-1 signaling pathway, providing an autocrine endothelin loop that may promote fibrosis in scleroderma [78]. The combination of increased endothelin in plasma in scleroderma [79], linked TGF- β -endothelin activation of fibroblast collagen synthesis, elevated endothelin-1 in scleroderma fibroblasts compared with normal fibroblasts, and endothelin receptor polymorphisms (see below) are proposed as evidence for the contribution of endothelin to fibrosis in scleroderma.

Vascular Injury

Vascular injury is a prominent feature of scleroderma. There is proliferation of vascular intima, and differentiation of smooth muscle cells and possibly monocytes to myofibroblasts causing narrowing of the vascular lumen. Several possible triggers of injury have been proposed, including environmental triggers such as cytomegalovirus or parvovirus that cause vessel damage [80, 81]. Possible immunologic triggers include cytokines (TGF- β , CTGF) or growth factors (PDGF, VEGF) that produce impaired vasodilation, leading to ischemia-reperfusion vascular injury. AECA (see above) may be directly pathogenic.

Genetic Factors

HLA Associations

Early studies in cohorts of individuals with scleroderma revealed no HLA associations. Later studies in which subgroups of individuals with certain autoantibody profiles were analyzed were more revealing [82, 83]. Examples of some of the associations are given below:

- Anti-topoisomerase antibody is associated with HLA DRB 1*1101/1104 and DPB 1*1301 alleles [54, 84].
- RNAP 1/III (RNA polymerase) autoantibodies are associated with DRB1*405, DRB4*01 and DQB1*0401 (Japanese), and DRB3*02 (Caucasians) [85].
- Anticentromere antibodies are associated with DRB1*01, DRB1*04 and DQB1*05 [54].
- There are also disproportionate increases in certain HLA alleles in diffuse scleroderma (DRB1*1104) and limited scleroderma (DRB1*1101). Both

diffuse and limited scleroderma have increased DRB1*11 compared to controls [86].

Of interest have been the twin studies which show low concordance of monozygotic and dizygotic twins for scleroderma (5%), but a high concordance for HLA haplotypes, suggesting that genetic factors alone are not enough for the development of scleroderma [87, 88]. There is infrequent familial aggregation of scleroderma (1–1.5%), again supporting predisposition but not causality of genetic factors [89].

Polymorphisms

The wide variety of genetic polymorphisms (table 3) suggests that there may be multiple pathways to autoimmune fibrosing disease. A certain immunologic or matrix phenotype may affect disease expression in an individual with a predisposition to scleroderma. The genetic polymorphisms occur not only in the HLA molecules, but also in cytokines and chemokines, and in vascular and extracellular matrix molecules.

Microchimerism of Fetal/Maternal Cells

In pregnancy, the placenta allows two-way exchange of fetal and maternal cells. The result is microchimerism, which can persist for HLA-compatible stem cells and precursor immune cells for many years. For instance, CD34+ and CD34+CD68+ fetal cells are present in maternal circulation up to 27 years after a pregnancy [90]. Microchimerism has been proposed as a hypothesis for scleroderma [91–93] because (1) women with scleroderma are more likely to have had an HLA-compatible fetus than matched controls and (2) Y-chromosome-positive cells are found in skin of women with scleroderma many years after childbirth [94]. This hypothesis is still in debate because microchimerism is a common event in normal individuals as well as in those with autoimmune disease. Persistent fetal stem cells may in fact be beneficial rather than pathogenic, providing renewal stem cells and circulating to sites of inflammation to help with repair [95, 96].

Environmental Triggers

Multiple environmental agents, among them bleomycin, silica, vinyl chloride, epoxy resins, adulterated cooking oils, L-tryptophan contaminants and solvents, can produce autoimmune fibrosing disease [for summary, see 2, 97]. There are several intriguing reports of scleroderma associated with systemic therapy for other disorders, suggesting a predisposition to scleroderma that can be uncovered or exacerbated by manipulation of the cytokine environment. In one report, two individuals receiving IFN- α , one for chronic active hepatitis, developed diffuse scleroderma and lung fibrosis 6 months after initiation of the agent [98].

Table 3. Genetic polymorphisms in scleroderma

Molecule	Allele	Role	Association	Reference
<i>Cytokines and chemokines</i>				
IL-1 α promoter	– 889T	Proinflammatory	Lung disease and response to cyclophosphamide	[113, 114]
IL-6 promoter	– 597	Profibrotic	Disease activity	[115]
IL-10	– 3575 – 2763	Anti-inflammatory		[116]
IL-13	– 1055 Rs2243204T	Profibrotic		[117]
TNF- α promoter	– 863	Proinflammatory		[88, 118]
TNF- α receptor	– 238 + 489	Regulatory		
MCP-1 promoter	– 2518G	Proinflammatory		[119]
MIF-1	– 173C	Anti-inflammatory	Lower in limited scleroderma	[120]
<i>Immune cell markers</i>				
CD86 (B7.2) promoter	– 3479T	Antigen-presenting cell-T cell signaling		[121]
CTLA-4 promoter	– 1722C – 1661G – 318T	T cell signaling		[122]
CD19 promoter	– 499T 3' UT region (GT)(14)	Regulation of B cell immune responses		[123]
<i>Vascular and matrix molecules</i>				
Endothelin receptor A, B	Increased B-1a B-2a alleles; + 69 and + 105 of exon 6 of receptor A	Vasoconstriction, modulation and ECM turnover; promotes myofibroblast differentiation	Diffuse scleroderma RNA polymerase antibodies	[124]
NOS	– 186C 894T	Regulation of the microcirculation		[125, 126]
ACE	insertion/deletion	Regulation of the microcirculation	Diffuse scleroderma	[126]
Fibrillin	5' UT region of exon1	Component of microfibrils in ECM	Choctaw Indians, Japanese	[127, 128]

Table 3. (continued)

Molecule	Allele	Role	Association	Reference
Metallo-proteinases		ECM homeostasis		[129]
AIF-1	Isoform 2	Immune response and proliferative vasculopathy	Diffuse scleroderma	[130]

MIF-1 = Macrophage inhibitory factor; ECM = extracellular matrix; NOS = nitric oxide synthase; ACE = angiotensin-converting enzyme; UT = untranslated; AIF-1 = allograft inflammatory factor 1.

In summary, scleroderma is a complex, probably multigenic, chronic and progressive autoimmune disease. It may be the end point of many different pathological processes involving the immune system, vessels and extracellular matrix in individuals with a genetic predisposition. The course of disease is dependent on the scleroderma variant, which can be identified with autoantibody profiles as well as clinical parameters. Systemic sclerosis/scleroderma has the highest morbidity and mortality. Molecular advances such as gene arrays, and more sensitive methods and reagents for detection of molecules and cells of the immune system, vessels and extracellular matrix have increased the body of knowledge about scleroderma. However, except for a few agents for the vascular abnormalities in scleroderma, no definitive therapy is available to date.

In one way, scleroderma is like the elephant in the ancient story about the 6 blind men and the elephant. Each blind man thought that the elephant was like the body part that he touched (such as the trunk, tail and foot). The elephant driver said to the blind men: 'Even if you put all the parts you can feel together, still it will not be a complete elephant. Many parts are still missing. As you have seen, you cannot see the complete elephant by putting together parts. You must see the whole elephant to have a complete idea of the elephant' [99]. For scleroderma, the whole elephant has not yet been seen and the diverse hypotheses about pathophysiology are summed up in this poem:

O how they cling and wrangle, some who claim
For preacher and monk the honored name!
For, quarreling, each to his view they cling.
Such folk see only one side of a thing [99].

Fortunately the scleroderma community of investigators has a very productive forum for exchange of ideas and data every two years at the International Scleroderma Workshop, organized by Drs. Carol Black (Royal Free Hospital) and the late Joseph Korn (Boston University), and now by Robert Lafayatis (Boston University). Together, this group of researchers, clinicians and industry

sponsors has moved the field forward toward a better understanding of the pathophysiology of scleroderma and toward better diagnosis and treatment of individuals with scleroderma.

References

- 1 Mayes MD, Lacey JV Jr, 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;48:2246–2255.
- 2 Nietert PJ, Silver RM: Systemic sclerosis: environmental and occupational risk factors. *Curr Opin Rheumatol* 2000;12:520–526.
- 3 Gardner H, Shearstone JR, Bandaru R, Crowell T, Lynes M, Trojanowska M, Pannu J, Smith E, Jablonska S, Blaszczyk M, Tan FK, Mayes MD: Gene profiling of scleroderma skin reveals robust signatures of disease that are imperfectly reflected in the transcript profiles of explanted fibroblasts. *Arthritis Rheum* 2006;54:1961–1973.
- 4 Lakos G, Takagawa S, Varga J: Animal models of scleroderma. *Methods Mol Med* 2004;102:377–393.
- 5 Zhang Y, Gilliam AC: Animal models for scleroderma: an update. *Curr Rheumatol Rep* 2002;4:150–162.
- 6 Abraham DJ, Varga J: Scleroderma: from cell and molecular mechanisms to disease models. *Trends Immunol* 2005;26:587–595.
- 7 Yamamoto T: The bleomycin-induced scleroderma model: what have we learned for scleroderma pathogenesis? *Arch Dermatol Res* 2006;297:333–344.
- 8 Yamamoto T, Nishioka K: Cellular and molecular mechanisms of bleomycin-induced murine scleroderma: current update and future perspective. *Exp Dermatol* 2005;14:81–95.
- 9 Ruzek MC, Jha S, Ledbetter S, Richards SM, Garman RD: A modified model of graft-versus-host-induced systemic sclerosis (scleroderma) exhibits all major aspects of the human disease. *Arthritis Rheum* 2004;50:1319–1331.
- 10 Anderson BE, McNiff JM, Jain D, Blazar BR, Shlomchik WD, Shlomchik MJ: Distinct roles for donor- and host-derived antigen-presenting cells and costimulatory molecules in murine chronic graft-versus-host disease: requirements depend on target organ. *Blood* 2005;105:2227–2234.
- 11 Kaplan DH, Anderson BE, McNiff JM, Jain D, Shlomchik MJ, Shlomchik WD: Target antigens determine graft-versus-host disease phenotype. *J Immunol* 2004;173:5467–5475.
- 12 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;163:5693–5699.
- 13 Zhang Y, McCormick LL, Desai SR, Wu C, Gilliam AC: Murine sclerodermatous graft-versus-host disease, a model for human scleroderma: cutaneous cytokines, chemokines, and immune cell activation. *J Immunol* 2002;168:3088–3098.
- 14 Zhang Y, McCormick LL, Gilliam AC: Latency-associated peptide prevents skin fibrosis in murine sclerodermatous graft-versus-host disease, a model for human scleroderma. *J Invest Dermatol* 2003;121:713–719.
- 15 Zhou L, Askew D, Wu C, Gilliam AC: Cutaneous gene expression by DNA microarray in murine sclerodermatous graft-versus-host disease, a model for human scleroderma. *J Invest Dermatol* 2006.
- 16 Gilliam AC: Experimental mouse models for scleroderma; in Chan LS (ed): *Animal Models of Human Inflammatory Skin Diseases*. Boca Raton, CRC Press, 2004, pp 517–534.
- 17 Worda M, Sgonc R, Dietrich H, Niederegger H, Sundick RS, Gershwin ME, Wick G: In vivo analysis of the apoptosis-inducing effect of anti-endothelial cell antibodies in systemic sclerosis by the chorionallantoic membrane assay. *Arthritis Rheum* 2003;48:2605–2614.
- 18 Prelog M, Scheidegger P, Peter S, Gershwin ME, Wick G, Sgonc R: Diminished transforming growth factor β 2 production leads to increased expression of a profibrotic procollagen α 2 type I

- messenger RNA variant in embryonic fibroblasts of UCD-200 chickens, a model for systemic sclerosis. *Arthritis Rheum* 2005;52:1804–1811.
- 19 Christner PJ, Jimenez SA: Animal models of systemic sclerosis: insights into systemic sclerosis pathogenesis and potential therapeutic approaches. *Curr Opin Rheumatol* 2004;16:746–752.
 - 20 Denton CP, Abraham DJ: Transgenic analysis of scleroderma: understanding key pathogenic events in vivo. *Autoimmun Rev* 2004;3:285–293.
 - 21 Sonnylal S, Denton CP, Zheng B, Keene DR, He R, Adams HP, Vanpelt CS, Geng YJ, Deng JM, Behringer RR, de Crombrughe B: Postnatal induction of transforming growth factor β signaling in fibroblasts of mice recapitulates clinical, histologic, and biochemical features of scleroderma. *Arthritis Rheum* 2006;56:334–344.
 - 22 Chang HY, Chi JT, Dudoit S, Bondre C, van de Rijn M, Botstein D, Brown PO: Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc Natl Acad Sci USA* 2002;99:12877–12882.
 - 23 Derk CT, Jimenez SA: Systemic sclerosis: current views of its pathogenesis. *Autoimmun Rev* 2003;2:181–191.
 - 24 Sakkas LI: New developments in the pathogenesis of systemic sclerosis. *Autoimmunity* 2005;38:113–116.
 - 25 Steen V: Targeted therapy for systemic sclerosis. *Autoimmun Rev* 2006;5:122–124.
 - 26 Denton CP, Black CM, Abraham DJ: Mechanisms and consequences of fibrosis in systemic sclerosis. *Nat Clin Pract Rheumatol* 2006;2:134–144.
 - 27 Kissin EY, Korn JH: Fibrosis in scleroderma. *Rheum Dis Clin North Am* 2003;29:351–369.
 - 28 Pannu J, Trojanowska M: Recent advances in fibroblast signaling and biology in scleroderma. *Curr Opin Rheumatol* 2004;16:739–745.
 - 29 Zuber JP, Chizzolini C, Leimgruber A, Bart PA, Spertini F: Pathogenic mechanisms in systemic sclerosis and their therapeutical consequences. Part 1. Pathogenesis. *Rev Med Suisse* 2006;2:1052–1057.
 - 30 Arnett FC: Is scleroderma an autoantibody mediated disease? *Curr Opin Rheumatol* 2006;18:579–581.
 - 31 Pannu J, Gardner H, Shearstone JR, Smith E, Trojanowska M: Increased levels of transforming growth factor β receptor type I and up-regulation of matrix gene program: a model of scleroderma. *Arthritis Rheum* 2006;54:3011–3021.
 - 32 Charles C, Clements P, Furst DE: Systemic sclerosis: hypothesis-driven treatment strategies. *Lancet* 2006;367:1683–1691.
 - 33 Varga J: Antifibrotic therapy in scleroderma: extracellular or intracellular targeting of activated fibroblasts? *Curr Rheumatol Rep* 2004;6:164–170.
 - 34 Braun-Moscovici Y, Furst DE: Stem cell therapy in scleroderma. *Curr Opin Rheumatol* 2002;14:711–716.
 - 35 Merkel PA, Clements PJ, Reveille JD, Suarez-Almazor ME, Valentini G, Furst DE: Current status of outcome measure development for clinical trials in systemic sclerosis. Report from OMERACT 6. *J Rheumatol* 2003;30:1630–1647.
 - 36 Furst DE, Khanna D, Mattucci-Cerinic M, Silman AJ, Merkel PA, Foeldvari I: Scleroderma–developing measures of response. *J Rheumatol* 2005;32:2477–2480.
 - 37 Atamas SP, White B: Cytokine regulation of pulmonary fibrosis in scleroderma. *Cytokine Growth Factor Rev* 2003;14:537–550.
 - 38 Atamas SP, White B: The role of chemokines in the pathogenesis of scleroderma. *Curr Opin Rheumatol* 2003;15:772–777.
 - 39 Scala E, Pallotta S, Frezzolini A, Abeni D, Barbieri C, Sampogna F, De Pita O, Puddu P, Paganelli R, Russo G: Cytokine and chemokine levels in systemic sclerosis: relationship with cutaneous and internal organ involvement. *Clin Exp Immunol* 2004;138:540–546.
 - 40 Denton CP, Abraham DJ: Transforming growth factor- β and connective tissue growth factor: key cytokines in scleroderma pathogenesis. *Curr Opin Rheumatol* 2001;13:505–511.
 - 41 Bhogal RK, Stoica CM, McGaha TL, Bona CA: Molecular aspects of regulation of collagen gene expression in fibrosis. *J Clin Immunol* 2005;25:592–603.
 - 42 Simms RW, Korn JH: Cytokine directed therapy in scleroderma: rationale, current status, and the future. *Curr Opin Rheumatol* 2002;14:717–722.

- 43 Yamamoto T, Eckes B, Hartmann K, Krieg T: Expression of monocyte chemoattractant protein-1 in the lesional skin of systemic sclerosis. *J Dermatol Sci* 2001;26:133–139.
- 44 Hayakawa I, Hasegawa M, Matsushita T, Yanaba K, Koderä M, Komura K, Takehara K, Sato S: Increased cutaneous T-cell-attracting chemokine levels in sera from patients with systemic sclerosis. *Rheumatology (Oxford)* 2005;44:873–878.
- 45 Yamamoto T: Chemokines and chemokine receptors in scleroderma. *Int Arch Allergy Immunol* 2006;140:345–356.
- 46 Carulli MT, Ong VH, Ponticos M, Shiwen X, Abraham DJ, Black CM, Denton CP: Chemokine receptor CCR2 expression by systemic sclerosis fibroblasts: evidence for autocrine regulation of myofibroblast differentiation. *Arthritis Rheum* 2005;52:3772–3782.
- 47 Tan FK, Zhou X, Mayes MD, Gourh P, Guo X, Marcum C, Jin L, Arnett FC Jr: Signatures of differentially regulated interferon gene expression and vasculotrophism in the peripheral blood cells of systemic sclerosis patients. *Rheumatology (Oxford)* 2006;45:694–702.
- 48 Tedder TF, Poe JC, Fujimoto M, Haas KM, Sato S: The CD19-CD21 signal transduction complex of B lymphocytes regulates the balance between health and autoimmune disease: systemic sclerosis as a model system. *Curr Dir Autoimmun* 2005;8:55–90.
- 49 Hasegawa M, Fujimoto M, Takehara K, Sato S: Pathogenesis of systemic sclerosis: altered B cell function is the key linking systemic autoimmunity and tissue fibrosis. *J Dermatol Sci* 2005;39:1–7.
- 50 Fujimoto M, Sato S: B lymphocytes and systemic sclerosis. *Curr Opin Rheumatol* 2005;17:746–751.
- 51 Matsushita T, Hasegawa M, Yanaba K, Koderä M, Takehara K, Sato S: Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum* 2006;54:192–201.
- 52 Ho KT, Reveille JD: The clinical relevance of autoantibodies in scleroderma. *Arthritis Res Ther* 2003;5:80–93.
- 53 Harris ML, Rosen A: Autoimmunity in scleroderma: the origin, pathogenetic role, and clinical significance of autoantibodies. *Curr Opin Rheumatol* 2003;15:778–784.
- 54 Cepeda EJ, Reveille JD: Autoantibodies in systemic sclerosis and fibrosing syndromes: clinical indications and relevance. *Curr Opin Rheumatol* 2004;16:723–732.
- 55 Senecal JL, Henault J, Raymond Y: The pathogenic role of autoantibodies to nuclear autoantigens in systemic sclerosis (scleroderma). *J Rheumatol* 2005;32:1643–1649.
- 56 Chung L, Utz PJ: Antibodies in scleroderma: direct pathogenicity and phenotypic associations. *Curr Rheumatol Rep* 2004;6:156–163.
- 57 Asano Y, Ihn H, Yamane K, Kubo M, Tamaki K: The prevalence and clinical significance of anti-U1 RNA antibodies in patients with systemic sclerosis. *J Invest Dermatol* 2003;120:204–210.
- 58 Servettaz A, Tamby MC, Guilpain P, Reinbolt J, Garcia de la Pena-Lefebvre P, Allanore Y, Kahan A, Meyer O, Guillevin L, Mouthon L: Anti-endothelial cell antibodies from patients with limited cutaneous systemic sclerosis bind to centromeric protein B (CENP-B). *Clin Immunol* 2006;120: 212–219.
- 59 Wusirika R, Ferri C, Marin M, Knight DA, Waldman WJ, Ross P Jr, Magro CM: The assessment of anti-endothelial cell antibodies in scleroderma-associated pulmonary fibrosis: a study of indirect immunofluorescent and western blot analysis in 49 patients with scleroderma. *Am J Clin Pathol* 2003;120:596–606.
- 60 Henault J, Robitaille G, Senecal JL, Raymond Y: DNA topoisomerase I binding to fibroblasts induces monocyte adhesion and activation in the presence of anti-topoisomerase I autoantibodies from systemic sclerosis patients. *Arthritis Rheum* 2006;54:963–973.
- 61 Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, Fraticelli P, Sambo P, Funaro A, Kazlauskas A, Avvedimento EV, Gabrielli A: Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med* 2006;354:2667–2676.
- 62 Tan FK: Autoantibodies against PDGF receptor in scleroderma. *N Engl J Med* 2006;354:2709–2711.
- 63 Casciola-Rosen L, Wigley F, Rosen A: Scleroderma autoantigens are uniquely fragmented by metal-catalyzed oxidation reactions: implications for pathogenesis. *J Exp Med* 1997;185:71–79.
- 64 Rosen A, Casciola-Rosen L, Wigley F: Role of metal-catalyzed oxidation reactions in the early pathogenesis of scleroderma. *Curr Opin Rheumatol* 1997;9:538–543.
- 65 Strehlow D, Korn JH: Biology of the scleroderma fibroblast. *Curr Opin Rheumatol* 1998;10: 572–578.

- 66 Postlethwaite AE, Shigemitsu H, Kanangat S: Cellular origins of fibroblasts: possible implications for organ fibrosis in systemic sclerosis. *Curr Opin Rheumatol* 2004;16:733–738.
- 67 Trojanowska M: What did we learn by studying scleroderma fibroblasts? *Clin Exp Rheumatol* 2004;22:S59–S63.
- 68 Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R: Circulating fibrocytes: collagen-secreting cells of the peripheral blood. *Int J Biochem Cell Biol* 2004;36:598–606.
- 69 Rajkumar VS, Howell K, Csiszar K, Denton CP, Black CM, Abraham DJ: Shared expression of phenotypic markers in systemic sclerosis indicates a convergence of pericytes and fibroblasts to a myofibroblast lineage in fibrosis. *Arthritis Res Ther* 2005;7:R1113–R1123.
- 70 Kissin E, Korn JH: Apoptosis and myofibroblasts in the pathogenesis of systemic sclerosis. *Curr Rheumatol Rep* 2002;4:129–135.
- 71 Jelaska A, Korn JH: Role of apoptosis and transforming growth factor β 1 in fibroblast selection and activation in systemic sclerosis. *Arthritis Rheum* 2000;43:2230–2239.
- 72 Flanders KC: Smad3 as a mediator of the fibrotic response. *Int J Exp Pathol* 2004;85:47–64.
- 73 Mori Y, Chen SJ, Varga J: Expression and regulation of intracellular SMAD signaling in scleroderma skin fibroblasts. *Arthritis Rheum* 2003;48:1964–1978.
- 74 Dong C, Zhu S, Wang T, Yoon W, Li Z, Alvarez RJ, ten Dijke P, White B, Wigley FM, Goldschmidt-Clermont PJ: Deficient Smad7 expression: a putative molecular defect in scleroderma. *Proc Natl Acad Sci USA* 2002;99:3908–3913.
- 75 Shi-Wen X, Denton CP, Dashwood MR, Holmes AM, Bou-Gharios G, Pearson JD, Black CM, Abraham DJ: Fibroblast matrix gene expression and connective tissue remodeling: role of endothelin-1. *J Invest Dermatol* 2001;116:417–425.
- 76 Shi-Wen X, Chen Y, Denton CP, Eastwood M, Renzoni EA, Bou-Gharios G, Pearson JD, Dashwood M, du Bois RM, Black CM, Leask A, Abraham DJ: Endothelin-1 promotes myofibroblast induction through the ETA receptor via a rac/phosphoinositide 3-kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibrotic fibroblasts. *Mol Biol Cell* 2004;15:2707–2719.
- 77 Waters CE, Shi-Wen X, Denton CP, Abraham DJ, Pearson JD: Signaling pathways regulating intercellular adhesion molecule 1 expression by endothelin 1: comparison with interleukin-1 β in normal and scleroderma dermal fibroblasts. *Arthritis Rheum* 2006;54:649–660.
- 78 Shi-Wen X, Rodriguez-Pascual F, Lamas S, Holmes A, Howat S, Pearson JD, Dashwood MR, du Bois RM, Denton CP, Black CM, Abraham DJ, Leask A: Constitutive ALK5-independent c-Jun N-terminal kinase activation contributes to endothelin-1 overexpression in pulmonary fibrosis: evidence of an autocrine endothelin loop operating through the endothelin A and B receptors. *Mol Cell Biol* 2006;26:5518–5527.
- 79 Kahaleh MB: Endothelin, an endothelial-dependent vasoconstrictor in scleroderma. Enhanced production and profibrotic action. *Arthritis Rheum* 1991;34:978–983.
- 80 Hamamdziec D, Kasman LM, LeRoy EC: The role of infectious agents in the pathogenesis of systemic sclerosis. *Curr Opin Rheumatol* 2002;14:694–698.
- 81 Magro CM, Crowder AN, Ferri C: Cytomegalovirus-associated cutaneous vasculopathy and scleroderma sans inclusion body change. *Hum Pathol* 2007;38:42–49.
- 82 Arnett FC: HLA and autoimmunity in scleroderma (systemic sclerosis). *Int Rev Immunol* 1995;12:107–128.
- 83 Johnson RW, Tew MB, Arnett FC: The genetics of systemic sclerosis. *Curr Rheumatol Rep* 2002;4:99–107.
- 84 Grutters JC, du Bois RM: Genetics of fibrosing lung diseases. *Eur Respir J* 2005;25:915–927.
- 85 Kuwana M, Pandey JP, Silver RM, Kawakami Y, Kaburaki J: HLA class II alleles in systemic sclerosis patients with anti-RNA polymerase I/III antibody: associations with subunit reactivities. *J Rheumatol* 2003;30:2392–2397.
- 86 Loubiere LS, Lambert NC, Madeleine MM, Porter AJ, Mullarkey ME, Pang JM, Galloway DA, Furst DE, Nelson JL: HLA allelic variants encoding DR11 in diffuse and limited systemic sclerosis in Caucasian women. *Rheumatology (Oxford)* 2005;44:318–322.
- 87 Feghali-Bostwick C, Medsger TA Jr, Wright TM: Analysis of systemic sclerosis in twins reveals low concordance for disease and high concordance for the presence of antinuclear antibodies. *Arthritis Rheum* 2003;48:1956–1963.

- 88 Tulusso B, Fabris M, Caporali R, Cuomo G, Isola M, Soldano F, Montecucco C, Valentini G, Ferraccioli G: -238 and +489 TNF- α along with TNF-RII gene polymorphisms associate with the diffuse phenotype in patients with systemic sclerosis. *Immunol Lett* 2005;96:103–108.
- 89 Tan FK, Arnett FC: Genetic factors in the etiology of systemic sclerosis and Raynaud phenomenon. *Curr Opin Rheumatol* 2000;12:511–519.
- 90 Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA: Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci USA* 1996;93:705–708.
- 91 Johnson KL, Nelson JL, Furst DE, McSweeney PA, Roberts DJ, Zhen DK, Bianchi DW: Fetal cell microchimerism in tissue from multiple sites in women with systemic sclerosis. *Arthritis Rheum* 2001;44:1848–1854.
- 92 Nelson JL, Furst DE, Maloney S, Gooley T, Evans PC, Smith A, Bean MA, Ober C, Bianchi DW: Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. *Lancet* 1998;351:559–562.
- 93 Artlett CM: Microchimerism and scleroderma: an update. *Curr Rheumatol Rep* 2003;5:154–159.
- 94 Artlett CM, Cox LA, Jimenez SA: Detection of cellular microchimerism of male or female origin in systemic sclerosis patients by polymerase chain reaction analysis of HLA-Cw antigens. *Arthritis Rheum* 2000;43:1062–1067.
- 95 Jimenez SA, Artlett CM: Microchimerism and systemic sclerosis. *Curr Opin Rheumatol* 2005;17:86–90.
- 96 Gilliam AC: Microchimerism and skin disease: true-true unrelated? *J Invest Dermatol* 2006;126:239–241.
- 97 Magnant J, de Monte M, Guilmet JL, Lasfargues G, Diot P, Asquier E, Degenne D, Boissinot E, Diot E: Relationship between occupational risk factors and severity markers of systemic sclerosis. *J Rheumatol* 2005;32:1713–1718.
- 98 Solans R, Bosch JA, Esteban I, Vilardell M: Systemic sclerosis developing in association with the use of interferon α therapy for chronic viral hepatitis. *Clin Exp Rheumatol* 2004;22:625–628.
- 99 Udana 68–69: Parable of the Blind Men and the Elephant.
- 100 Zhou X, Tan FK, Milewicz DM, Guo X, Bona CA, Arnett FC: Autoantibodies to fibrillin-1 activate normal human fibroblasts in culture through the TGF- β pathway to recapitulate the ‘scleroderma phenotype’. *J Immunol* 2005;175:4555–4560.
- 101 Pandey JP, Page GP, Silver RM, LeRoy EC, Bona CA: Anti-fibrillin-1 autoantibodies in systemic sclerosis are GM and KM allotype-restricted. *Exp Clin Immunogenet* 2001;18:123–129.
- 102 Sato S, Hayakawa I, Hasegawa M, Fujimoto M, Takehara K: Function blocking autoantibodies against matrix metalloproteinase-1 in patients with systemic sclerosis. *J Invest Dermatol* 2003;120:542–547.
- 103 Nishijima C, Hayakawa I, Matsushita T, Komura K, Hasegawa M, Takehara K, Sato S: Autoantibody against matrix metalloproteinase-3 in patients with systemic sclerosis. *Clin Exp Immunol* 2004;138:357–363.
- 104 Jinnin M, Ihn H, Asano Y, Yamane K, Yazawa N, Tamaki K: Serum matrix metalloproteinase-3 in systemic sclerosis. *Arch Dermatol Res* 2004;296:25–29.
- 105 Yasuoka H, Ihn H, Medsger TA Jr, Hirakata M, Kawakami Y, Ikeda Y, Kuwana M: A novel protein highly expressed in testis is overexpressed in systemic sclerosis fibroblasts and targeted by autoantibodies. *J Immunol* 2003;171:6883–6890.
- 106 Goto M, Okawa-Takatsuji M, Aotsuka S, Nakai H, Shimizu M, Goto H, Shimamoto A, Furuichi Y: Significant elevation of IgG anti-WRN (RecQ3 RNA/DNA helicase) antibody in systemic sclerosis. *Mod Rheumatol* 2006;16:229–234.
- 107 Yang JM, Hildebrandt B, Luderschmidt C, Pollard KM: Human scleroderma sera contain autoantibodies to protein components specific to the U3 small nucleolar RNP complex. *Arthritis Rheum* 2003;48:210–217.
- 108 Gilliam AC, Steitz JA: Rare scleroderma autoantibodies to the U11 small nuclear ribonucleoprotein and to the trimethylguanosine cap of U small nuclear RNAs. *Proc Natl Acad Sci USA* 1993;90:6781–6785.
- 109 Ulanet DB, Wigley FM, Gelber AC, Rosen A: Autoantibodies against B23, a nucleolar phosphoprotein, occur in scleroderma and are associated with pulmonary hypertension. *Arthritis Rheum* 2003;49:85–92.

- 110 Fujimoto M, Hamaguchi Y, Yazawa N, Komura K, Takehara K, Sato S: Autoantibodies to a collagen-specific molecular chaperone, heat-shock protein 47, in systemic sclerosis. *Clin Exp Immunol* 2004;138:534–539.
- 111 Spinette S, Lengauer C, Mahoney JA, Jallepalli PV, Wang Z, Casciola-Rosen L, Rosen A: Ufd2, a novel autoantigen in scleroderma, regulates sister chromatid separation. *Cell Cycle* 2004;3:1638–1644.
- 112 Hasegawa M, Sato S, Yanaba K, Komura K, Yamazaki M, Takehara K: Autoantibodies against phosphatidylserine-prothrombin complex in patients with systemic sclerosis. *Ann Rheum Dis* 2004;63:1514–1517.
- 113 Hutyrova B, Lukac J, Bosak V, Buc M, du Bois R, Petrek M: Interleukin 1 α single-nucleotide polymorphism associated with systemic sclerosis. *J Rheumatol* 2004;31:81–84.
- 114 Beretta L, Cappiello F, Barili M, Bertolotti F, Scorza R: T-889C IL-1 α promoter polymorphism influences the response to oral cyclophosphamide in scleroderma patients with alveolitis. *Clin Rheumatol* 2007;26:88–91.
- 115 Sfrent-Cornateanu R, Mihai C, Balan S, Ionescu R, Moldoveanu E: The IL-6 promoter polymorphism is associated with disease activity and disability in systemic sclerosis. *J Cell Mol Med* 2006;10:955–959.
- 116 Hudson LL, Rocca KM, Kuwana M, Pandey JP: Interleukin-10 genotypes are associated with systemic sclerosis and influence disease-associated autoimmune responses. *Genes Immun* 2005;6:274–278.
- 117 Granel B, Chevillard C, Allanore Y, Arnaud V, Cabantous S, Marquet S, Weiller PJ, Durand JM, Harle JR, Grange C, Frances Y, Berbis P, Gaudart J, de Micco P, Kahan A, Dessein A: Evaluation of interleukin 13 polymorphisms in systemic sclerosis. *Immunogenetics* 2006;58:693–699.
- 118 Sato H, Lagan AL, Alexopoulou C, Vassilakis DA, Ahmad T, Pantelidis P, Veeraghavan S, Renzoni E, Denton C, Black C, Wells AU, du Bois RM, Welsh KI: The TNF-863A allele strongly associates with antinuclear antibody positivity in scleroderma. *Arthritis Rheum* 2004;50:558–564.
- 119 Karrer S, Bosserhoff AK, Weiderer P, Distler O, Landthaler M, Szeimies RM, Muller-Ladner U, Scholmerich J, Hellerbrand C: The –2518 promotor polymorphism in the MCP-1 gene is associated with systemic sclerosis. *J Invest Dermatol* 2005;124:92–98.
- 120 Wu SP, Leng L, Feng Z, Liu N, Zhao H, McDonald C, Lee A, Arnett FC, Gregersen PK, Mayes MD, Bucala R: Macrophage migration inhibitory factor promoter polymorphisms and the clinical expression of scleroderma. *Arthritis Rheum* 2006;54:3661–3669.
- 121 Abdallah AM, Renzoni EA, Anevlavis S, Lagan AL, Munkonge FM, Fonseca C, Black CM, Briggs D, Wells AU, Marshall SE, McHugh N, du Bois RM, Welsh KI: A polymorphism in the promoter region of the CD86 (B7.2) gene is associated with systemic sclerosis. *Int J Immunogenet* 2006;33:155–161.
- 122 Almasi S, Erfani N, Mojtahedi Z, Rajaei A, Ghaderi A: Association of CTLA-4 gene promoter polymorphisms with systemic sclerosis in Iranian population. *Genes Immun* 2006;7:401–406.
- 123 Tsuchiya N, Kuroki K, Fujimoto M, Murakami Y, Tedder TF, Tokunaga K, Takehara K, Sato S: Association of a functional CD19 polymorphism with susceptibility to systemic sclerosis. *Arthritis Rheum* 2004;50:4002–4007.
- 124 Fonseca C, Renzoni E, Sestini P, Pantelidis P, Lagan A, Bunn C, McHugh N, Welsh KI, Du Bois RM, Denton CP, Black C, Abraham D: Endothelin axis polymorphisms in patients with scleroderma. *Arthritis Rheum* 2006;54:3034–3042.
- 125 Assassi S, Mayes MD, McNearney T, Fischbach M, Reveille JD, Arnett FC, Tan FK: Polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in systemic sclerosis. *Am J Med* 2005;118:907–911.
- 126 Fatini C, Gensini F, Sticchi E, Battaglini B, Angotti C, Conforti ML, Generini S, Pignone A, Abbate R, Matucci-Cerinic M: High prevalence of polymorphisms of angiotensin-converting enzyme (I/D) and endothelial nitric oxide synthase (Glu298Asp) in patients with systemic sclerosis. *Am J Med* 2002;112:540–544.
- 127 Kodera T, Tan FK, Sasaki T, Arnett FC, Bona CA: Association of 5'-untranslated region of the Fibrillin-1 gene with Japanese scleroderma. *Gene* 2002;297:61–67.
- 128 Tan FK, Wang N, Kuwana M, Chakraborty R, Bona CA, Milewicz DM, Arnett FC: Association of fibrillin 1 single-nucleotide polymorphism haplotypes with systemic sclerosis in Choctaw and Japanese populations. *Arthritis Rheum* 2001;44:893–901.

- 129 Marasini B, Casari S, Zeni S, Turri O, Biondi ML: Stromelysin promoter polymorphism is associated with systemic sclerosis. *Rheumatology (Oxford)* 2001;40:475–476.
- 130 Del Galdo F, Maul GG, Jimenez SA, Artlett CM: Expression of allograft inflammatory factor 1 in tissues from patients with systemic sclerosis and in vitro differential expression of its isoforms in response to transforming growth factor beta. *Arthritis Rheum* 2006;54:2616–2625.

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Alopecia Areata

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Abstract

The pathogenesis of organ specific, cell mediated autoimmune alopecia areata (AA) has substantially progressed in the last decade. These advances are partly based upon advances in immunology and genetics, improved technological methodology in RNA, DNA, proteomics, and computer analyses, as well as the development of the C3H/HeJ mouse model of AA. The discovery that full thickness skin grafts transfer AA from C3H/HeJ mice that spontaneously develop AA to multiple non-affected C3H/HeJ mice greatly shortened the time of AA onset and provided many more affected mice in this highly reproducible model of AA. These methodological and genetic advances combine to form practical bases for identifying subtypes of human and mouse AA, characterizing disease mechanisms, improving currently available treatments, and developing new, more effective therapies. In the next decade even more exciting new insights into the pathogenesis of subtypes of human AA, their genetic bases, and therapy development will become available based on in-depth data on specific gene mutations and signaling pathways involved. Other organ specific autoimmune diseases will surely benefit from the rapid progress in understanding AA.

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Alopecia Areata in Humans

What is it and how do we define alopecia areata (AA)? AA is a nonscarring, often spontaneously resolving, unifocal patchy hair loss most commonly occurring on the scalp. AA may evolve quickly or slowly from a unifocal patch to multifocal, reticulated patches, to ophiasis hair loss at the inferior, posterior neck, or its inverse form, sisaifo type, with hair loss limited to the vertex, and finally to a complete loss of scalp hair (alopecia totalis, AT; fig. 1). A diffuse form of AA may occur acutely and can become widespread. Commonly seen in the chronic AA disease state, it can be difficult to diagnose as no annular



Fig. 1. Human AA. AA may evolve from a single small patch (*a*) to a large patch (*b*) that patients try to cover with a wig to hide. Lesions may involve the neck as an ophiasis lesion (*c*). Small patches of hair loss such as a figurate (*e*) or reticulated (*f*) appearance often precede total baldness, the AT variant (*g*). Hair loss of other nonscalp sites, such as eyebrows, eyelashes, beard and other body sites are characteristic of the alopecia universalis variant (not shown).

patches are recognizable. The more severe forms of AA may also show partial or complete hair loss from any hair-bearing body region (alopecia universalis, AU). At times it may be difficult to classify the precise form of AA as being either AT or AU as it is evolving or regressing. The severe forms that may be classified as AT/AU are far less common (7% of all cases) than patch-type AA. Though the extent of hair loss and the course of disease vary for each individual, more extensive and persistent alopecia is typically associated with initial onset of prepuberty [1].

To accurately diagnose the unifocal patch stage of AA or the diffuse form of AA may require cultures, scalp biopsy (fig. 2) and even blood tests to exclude tinea capitis, acute telogen effluvium, trichotillomania and even alopecia syphilitica. Annular AA lesions may resemble tinea capitis but are fungal culture negative, often spontaneously regrow without scarring and, in the active stage, have diagnostic microscopic features in biopsy specimens. The spontaneous resolution features of AA make it difficult to define the pathogenesis of specific forms of AA and document the most effective treatments for each subtype of AA.

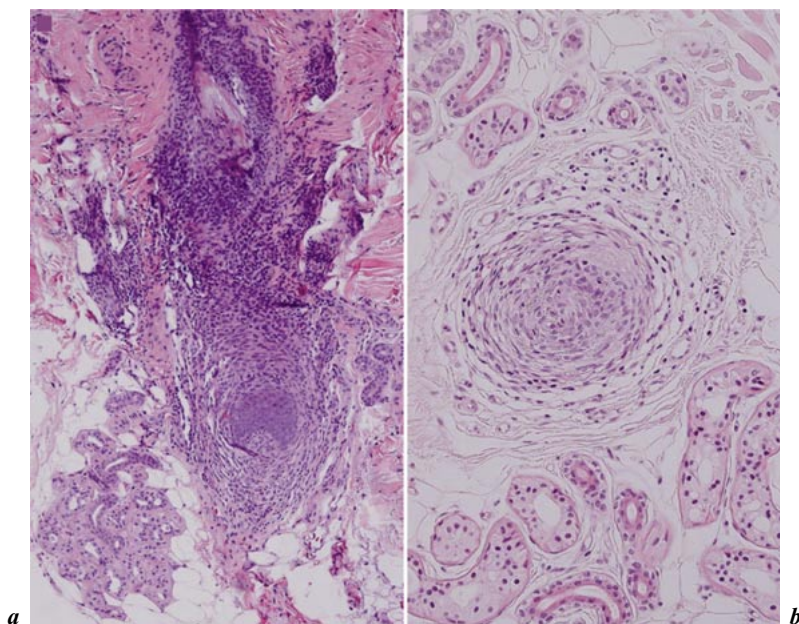


Fig. 2. Human AA. Histologic changes consist of infiltration in and around hair follicles as seen in this cross section (*a*) and extending from the bulb region to immediately below the sebaceous gland as seen in this vertical section (*b*).

Epidemiology of AA

Is AA rare and if so, how does its pathogenesis provide insight into other human diseases? In humans, AA occurs in 158 per 100,000 (0.1–0.2%) with a lifetime risk of 1.7% [2]. Approximately 1 in 5 patients with AA has another family member with AA. As an organ-specific autoimmune disorder, recent interest has been on defining its association with systemic and cutaneous autoimmune disorders as well as chronic inflammatory skin diseases. Between 7 and 27% of AA-affected patients may express a thyroid disease phenotype, including goiter presence, myxedema and Hashimoto's thyroiditis [3–7]. Coexpression of vitiligo and AA has also been reported [1, 8, 9]. However, some authors question whether the association of AA with thyroiditis and vitiligo is statistically significant [10, 11]. Numerous case reports detail concordant presence of AA with other autoimmune diseases, such as Addison's disease, pernicious anemia and myasthenia gravis, although the statistical significance is unknown [12]. An association of specific forms of AA with atopy in families, with atopic dermatitis, otitis, sinusitis, asthma and related conditions has also been suggested [13–16].

Early Theories of AA Pathogenesis

In the late nineteenth and early twentieth centuries, the clinical features of AA were thought to be due to an infectious etiology [17, 18] because of its focal nature, regrowth and recurrence at different sites, and its apparent occurrence in orphanages suggesting an ‘epidemic’ [19–21]. Virally induced immune reactions related to interferon-mediated pathways may still be relevant today (see below). However, to date no infectious agent(s) has been unequivocally identified in association with AA [22–24]. Although cytomegalovirus (CMV) induction or activation of AA [25, 26] was proposed previously, these data have been refuted [12, 27–30]. Despite the failure to identify infectious agents in AA, activation of the innate immune system by viral antigens is still a viable option to explain how AA and other organ-specific autoimmune diseases are initiated or regulated.

Other investigators in the late nineteenth century proposed that AA may be initiated by abnormalities in the central and peripheral nervous system (neurotrophic theory). The apparent induction of circumscribed alopecia after sectioning of the cervical ganglion in cats and cases of human alopecia where affected areas corresponded to specific nerve distribution were used as evidence to further support the idea of nervous system involvement in AA [18, 31, 32]. Recent evidence showing that the equivalent of a pituitary-adrenal axis system is present in the skin and affects cutaneous inflammatory and immune responses [33] continues to make this hypothesis potentially viable for some AA subtypes. A clinical history of emotional/physical stress or trauma preceding the onset of AA has been frequently noted [34]. While the potential for nervous system involvement in AA remains [35, 36], and stress is questioned as a potential trigger for onset of hair loss [37], the central neurotrophic hypothesis has much less support among dermatologists today.

Other hypotheses to explain the pathogenesis of AA included toxic agents, particularly with reports of thallium acetate (rat poison) injection apparently inducing patchy hair loss and exclamation mark-type hairs [23, 38, 39, 40]. Endocrine dysfunction was also suggested as a cause when AA was recognized in association with thyroid disease and hormonal fluctuations during pregnancy or menopause [39, 41–45]. Recent interest in the association of autoimmune polyendocrinopathy, mucocutaneous candidiasis, ectodermal defects (APECED) with AA has renewed interest in the potential for combined endocrine and immune dysfunction in this rare inherited condition [46].

Currently, while there is still significant debate as to the mechanisms of AA development, all hypotheses of how AA is induced and maintained in a chronic state have dysregulation of the immune system in common. Surprisingly, leukocyte inflammation of dystrophic AA-affected hair follicles was identified over one hundred years ago [47], but the hypothesis that inflammation may be responsible for AA did not gain widespread support until relatively recently. The

current concept that AA is an organ-specific autoimmune disease evolved from observations in the late 1950s [48]. Current research is now focused on which signaling pathways, cytokines and lymphocyte costimulatory pathways initiate or regulate severity, chronicity and susceptibility of AA as an organ-specific autoimmune disease (see below) [49–51].

AA in Humans Is an Autoimmune Inflammatory Disease

Cell-Mediated Immunity in AA

If AA is an organ-specific autoimmune disease, is the predominant immune dysfunction cellular, humoral or both? AA as an organ-specific autoimmune disease is currently the most widely accepted hypothesis [52–55]. In evolving AA, histologically apparent peri- and intrafollicular inflammation of the lower portions of only anagen hair follicles, primarily by T lymphocytes, is a common characteristic in both humans and animal models (fig. 2) [48, 56–59]. Other types of inflammatory cells functioning as antigen-presenting cells (APCs) are present around and within dystrophic hair follicles, particularly an increased number of macrophages and Langerhans cells [60]. Circumstantially, this inflammatory infiltrate is consistent with a targeted immune response against hair follicle-located antigens.

Why are specific sites of the human hair follicle in a specific stage of hair follicle growth interesting clues to the pathogenesis of AA? Inflammation of the lower aspects of hair follicles is a significant histological and diagnostic feature of human AA. One explanation for this feature may be a consequence of the transient immune privilege properties that hair follicles may have in the active anagen growing phase [61, 62]. Part of this immune privilege may come from the near absence of major histocompatibility complex (MHC) class I or II expression in normal hair follicles [63]. Abnormal expression of MHC class I and II antigens by AA that affected dystrophic anagen follicles has been documented [64–66], suggesting their aberrant expression in AA is a critical component in the follicular inflammation process of AA [67]. In evaluation of the cutaneous inflammatory responses in AA, studies document an upregulation of intercellular adhesion molecule (ICAM-1) and endothelial cell selectin (SELE, formerly ELAM) expression on the blood vessel endothelium closely associated with affected hair follicles [58, 68, 69]. Other cutaneous inflammatory changes in AA include increased production of proinflammatory cytokines such as interleukin-2 (IL-2) and interferon- γ (IFN- γ) that are ameliorated after successful topical counter irritant therapy [70].

Humoral Immunity in AA

Are the humoral antibody responses to hair follicle-related antigens the initiating event in inducing and/or chronically maintaining AA? Hair follicle-specific

IgG autoantibody titers in humans with AA are increased in the peripheral blood compared to normal, nonaffected humans [71, 72]. Similarly, hair follicle-specific autoantibodies with cross-species specificity to hair follicle antigens were detected in humans, dogs, horses, mouse and rat models of AA [12, 73–78]. Targeting of hair follicles in multiple mammalian species with AA-like alopecia by cross-reacting antibodies is persuasive circumstantial evidence of autoimmunity, but does not document that the humoral-induced autoantibodies are the primary disease-inducing response. In general, human humoral autoantibody-based autoimmune diseases are less prevalent than cell-mediated autoimmune diseases. In humans, rats and mice with an AA-like alopecia, autoantibodies to hair follicles do not consistently target the same or similar specific epitopes. Even though 75% of sera from human patients with AA have hair follicle-specific autoantibodies, almost 30% of sera from healthy humans also contain low levels of hair follicle-specific autoantibodies [71, 72]. Although the majority of human AA patients may have detectable anti-hair follicle IgG compared to controls, the major difference appears to be that human control sera only react with a single band, whereas AA patients' sera identify multiple bands with greater intensity in immunoblots using hair follicle extracts [Tobin, pers. commun.]. These data imply that there are multiple epitopes targeted by autoantibodies in the pathogenesis of AA and epitope targeting varies between individuals. This cannot easily be reconciled with the humoral response being the primary cause of AA. Other evidence also indicates that autoantibodies are not the predominant factor in AA disease development. For example, a patient with hypogammaglobulinemia who developed AA suggests autoantibodies are not fundamentally necessary for AA to develop [79]. Also, CBA/CaHN-*Btk^{xid}*/J mice with X-linked immunodeficiency are deficient in IgM and IgG3, but still develop an AA-like disease [80]. Further evidence that autoantibody production is a secondary, nonspecific event in the evolution of AA was provided by gene array studies showing an upregulation of a wide assortment of immunoglobulin genes relatively late in the disease process (see below) [81].

If autoantibody production is common in all mammalian species with an AA-like disease [82], how does this immune response affect the onset, severity or duration of AA? How do we explain that IgG autoantibodies are produced more often and in higher titers in patients affected with AA than in nonaffected individuals? Do different autoantibody clones affect differing harmful events, such as modifying severity, or do they have beneficial effects on specific features of AA [83]? For example, autoantibodies may be beneficial by helping to opsonize and clear antigenic debris [84]. A harmful effect of autoantibody generation against specific melanocyte-associated T cell epitopes appears to be involved in the transfer of AA in a xenograft model [85]. However, autoantibody

Table 1. Purported loci for human AA susceptibility genes and mouse orthologs

Human Gene	Human chromosome	Reference	Mouse Gene	Mouse chromosome ¹
<i>FCRL3</i>	1	192	<i>Fcrl5</i>	3
<i>HLA DQ</i>	6	107–109, 116, 119, 193–196	<i>H2-Ab1</i>	17; 18.64 cM
<i>HLA DR</i>	6	107, 116, 119, 193, 197	<i>H2-Eb2</i>	17; 18.67 cM
<i>IL1RN</i>	2	198	<i>Il1rn</i>	2; 10.0 cM
<i>IL1B</i>	2	199, 200	<i>IL1b</i>	2; 73.0 cM
<i>IL1</i>	2	200, 201	<i>IL1a</i>	2; 73.0 cM
<i>Km1</i>	2	199, 202–204	<i>Igk</i>	6; 30.0 cM
<i>MICA*5</i>		119		
<i>MICA*6</i>		119		
<i>MXI</i>	21	205	<i>Mx1</i>	16; 71.2 cM
<i>TNFA</i>	6	183	<i>Tnf</i>	17; 19.06 cM

¹Mouse Genome Informatics (www.informatics.jax.org, accessed 26 Jan 2007).

production against melanocyte-associated antigens has not been identified so far in AA. These studies are consistent with AA gene array data showing responses to melanin-related proteins were late in disease development and nonspecific [81].

Genetics of Human AA

Is the predisposition to AA genetic and if so what factor(s) are inherited? Previous studies have shown that at least some forms of AA are inherited [86–88], as AA occurs in identical twins [89–94], siblings [95] and in multiple generations of family members with AA [87, 96, 97]. A strong association between AA and trisomy 21 (Down syndrome) has been observed [98, 99]. Mutation in the autoimmune regulator gene (*AIRE*) results in the autoimmune polyendocrinopathy syndrome type 1, which is also associated with a 29–37% prevalence of AA [100]. These observations circumstantially suggest a role for genes on chromosome 21 in AA susceptibility (table 1).

To identify the inherited factors predisposing to human AA, the focus has primarily been on immune-related genes such as HLA-D. This chromosomal location is thought to be the most likely region for autoimmune-related genes regulating susceptibility or resistance to AA as an autoimmune disease (table 1). This is a reasonable assumption as other autoimmune diseases are associated with specific MHC class II haplotypes [88, 101] and certain haplotypes appear to be associated with a general predisposition to autoimmunity [96, 102–109].

AA may have MHC class II restriction [110–116]. AA has been shown to be associated with several MHC class II alleles, HLA-DQ3, HLA-DR4 and HLA-DR11. Alleles DQB1*03 and DRB1*1104 are significantly associated with all forms of AA, while DRB1*1104, DRB1*0401 and DQB1*0301 are specifically associated with long-standing and/or extensive AT and AU [117, 118]. Recently, using specimens from multiplex families ascertained by the National Alopecia Areata Registry, MICA*5 was found to be associated with patchy AA and MICA*6 associated with all forms of AA [119]. The reason for autoimmune disease association with certain MHC genes has not been elucidated, but may be a consequence of the molecular and structural alteration of the HLA peptide-binding site and/or a general predisposition to overexpression of HLA antigens on target tissue and APCs [120]. In support of this hypothesis, significant linkage was found in the mouse AA model in the homologous region of the mouse MHC [81, 121].

Is AA a single-organ defect in the predisposition to autoimmunity or is AA associated with genetic susceptibility to other autoimmune diseases in a patient and/or their family? The increased epidemiological association of human AA with other autoimmune diseases within the same individual and/or within blood relatives of affected people provides data suggesting inherited autoimmune susceptibility [1, 6, 122]. The success in using immunosuppressive or immunomodulatory treatments in some cases of AA [37, 123] also indicates that inflammation of the hair follicle is important in AA, hence the focus in human genetic studies on various immunoregulatory genes.

Animal Models for AA

Why has the progress to elucidate the causes and treatment of human AA been so slow? The variable onset, spontaneous resolution and simply the longevity of humans as an ‘outbred’ mammal make such research difficult. As animal models have been used extensively to study the etiology, complications and therapy of other human diseases, finding an appropriate animal model for human AA has been an obvious goal. Hair loss syndromes in mammals have been observed over the years, but most have been poorly characterized. Documented hair loss syndromes similar to AA have been observed in dogs, cats, horses, cattle, nonhuman primates and even a feather loss syndrome in chickens (table 2) [124–129].

Inbred rodents are ideal to study for many human diseases as they are small, easy to maintain and the most genetically well-defined mammalian species. The Dundee experimental bald rat (DEBR) has many features of AA and has been used in a number of research studies [130–132]. The HLA-B27

Table 2. Spontaneous and induced animal models in various species that resemble AA in humans.

Species	Type of model	Human disease	Reference
C3H/HeJ mouse	Spontaneous	AA like	59, 80, 128, 135
C3H/HeJ mouse	Graft induced	AA like	135
C3H/HeJBir mouse	Spontaneous	AA like	80
C3H/HeNJ mouse	Spontaneous	AA like	80
C3H/HeOuJ mouse	Spontaneous	AA like	80
BALB.2R- <i>H2^{h2}</i> /Lil mouse	Spontaneous	AA like	80
A/J Mouse	Spontaneous	AA like	80
CBA/CaHN- <i>Btk^{xid}</i> /J mouse	Spontaneous	AA like	80
HRS/J +/+ mouse	Spontaneous	AA like	80
HRS/J <i>hr/hr</i> mouse	Spontaneous	Papular atrichia	143, 151
C.B17 <i>Prkdc^{scid}</i> mouse	Xenograft	AA like	165, 206
CD18 null mouse	Targeted Mutation	Some features of AA and psoriasis	154
Dundee experimental bald rat (DEBR)	Spontaneous	AA like	128, 135
HLA-B27 rat	Transgenic	Some features of AA and psoriasis	133
Domestic dogs	Spontaneous	AA like	128, 135, 207, 208, 209
Horses	Spontaneous	AA like	210, 213
Cattle	Spontaneous	AA like	126, 127, 213
Chicken	Spontaneous	Autoimmune feather disease	129, 211, 212

transgenic rat develops AA-like and psoriasis-like skin diseases [133]. This transgenic rat model supports the findings that the HLA region of the human genome is a likely site for some genes involved in the pathogenesis of AA. However, the degree of similarity to human AA and the severity of the peri- and interfollicular lymphocytic infiltrates have not been described in detail. We reviewed this HLA-B27 rat model in unrelated studies and found it to be unsatisfactory due to minimal perifollicular lymphocytic infiltrates [Carroll and Sundberg, unpubl. observations]. While rat models of AA may be practical for some forms of research, mouse models have become the predominant model species for most laboratory studies.

Spontaneous Development of AA-Like Hair Loss in Inbred C3H/HeJ Mice

What mouse model best fits the criteria to examine the pathogenesis, genetics and therapy of human AA? We identified a form of spontaneous hair



Fig. 3. Spontaneous AA in the C3H/HeJ laboratory mouse. The top female mouse had diffuse alopecia with a rudimentary ‘fuzz’ of defective hair fibers that break easily. The lower mouse is an age-matched female with a normal hair coat and no evidence of AA.

loss in aging C3H/HeJ mice that closely mimics that seen in human AA [59, 75, 81, 128, 134, 135]. Patchy areas of hair loss on the back and larger, circumscribed areas on the abdomen consistently develop in up to 20% of female C3H/HeJ mice by 12 months of age (fig. 3). Similar lesions develop at a later age in male mice [59, 134]. In humans with AA, there is an equal or greater female to male ratio, or a two-fold excess in cases of affected females according to different authors [1, 2, 136]. Areas of alopecia on the dorsal skin wax and wane in severity as with human AA, and often become more generalized as occurs in human AT or AU. Histologically, there is a mixed, but predominantly mononuclear, cell infiltrate in and around anagen hair follicles in mice, as is the case in human AA (figs 4, 5). The infiltrate is localized and does not affect telogen follicles. Club hairs and exclamation point hair shafts are present, and both hair bulb melanocytes and keratinocytes are damaged as in human AA [137, 138]. Compounds commonly used to treat human AA (corticosteroids, squaric acid dibutylester, diphencyprone and tacrolimus) are also therapeutically effective in this AA mouse model [59, 139–142]. Lastly, as described below, mouse AA is associated with an abnormal antibody response to hair follicles as occurs in humans with AA [75].

Other Inbred Mouse Models for Human AA – Multiple Phenotypes?

What other mouse strains develop spontaneous diseases that resemble human AA? Inbred laboratory mice are the best model system to study mammalian

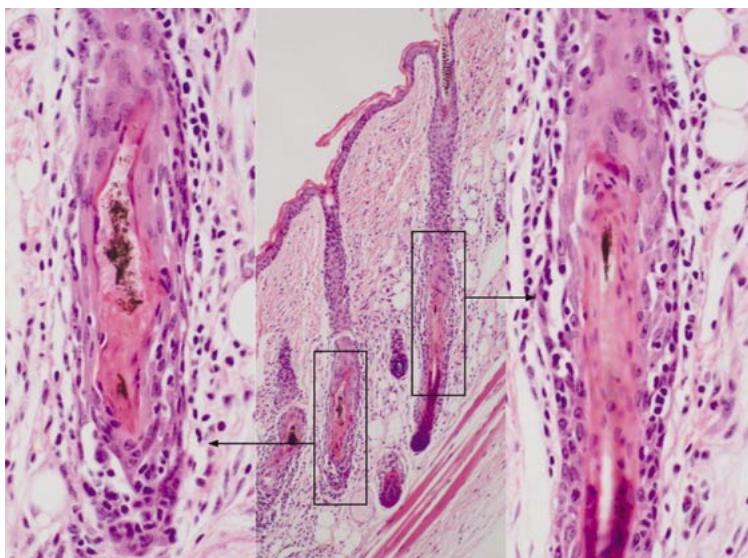


Fig. 4. Spontaneous AA in the C3H/HeJ laboratory mouse. Longitudinal sections of anagen stage hair follicles illustrate the different lengths of the guard (long) versus zigzag (higher location of a bulb at the dermal junction) hairs, not to be confused with miniaturization of follicles. Above the bulb, where MHC I is abnormally upregulated (comparable to the bulb and above in humans), there is infiltration in and around follicles by a variety of inflammatory cells, predominantly lymphocytes. These cells disrupt the function of the root sheaths resulting in formation of dystrophic fibers (higher magnification of boxed areas), which break when they reach the surface resulting in alopecia.

genetics, but progress in understanding the pathogenesis and genetics of AA and developing new therapies was severely hampered until the relatively recent discovery of suitable disease models. We retrospectively and prospectively evaluated a variety of mutant mice at The Jackson Laboratory with various forms of alopecia. Some of these mutants have been investigated and found to have a totally different disease compared to AA. For example hairless (*Hr*), originally touted as being AU (variant of AA) [143], was subsequently identified as a model for papular atrichia [144–152]. Another mutation arose spontaneously in our C3H/HeJ production colony that appeared to have a juvenile-onset AA-like clinical disease. This mutation (juvenile alopecia, *jal*) was characterized and the locus mapped to mouse chromosome 13 [153]. Comparative studies with the spontaneous form of AA in C3H/HeJ mice and with human AA cases revealed that *jal/jal* mice did not have a form of AA.

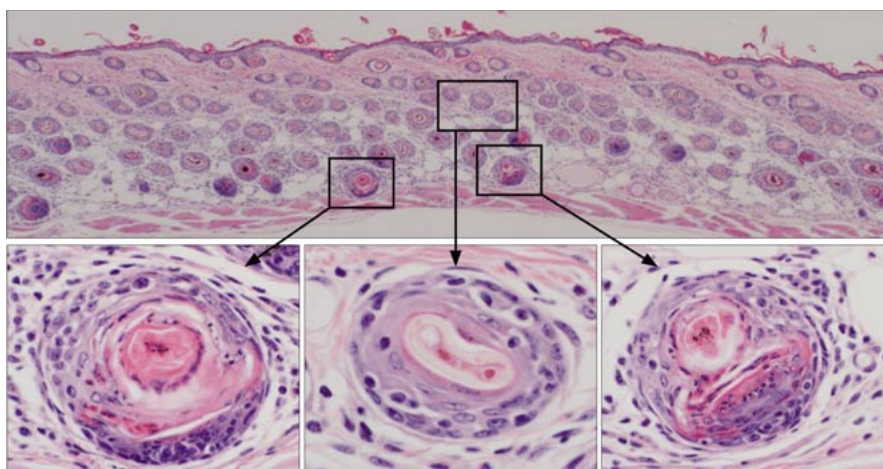


Fig. 5. Spontaneous AA in the C3H/HeJ laboratory mouse. Oblique histologic sections reveal the diffuse nature of the histologic lesions, inflammation in and around lower hair follicle (boxed area left and right) causing abnormal fiber production, and inflammation in and around follicle section extending into the dermis (boxed area middle).

Several different mouse strains with genetic mutations, targeted and spontaneous, have been examined for potential similarities to human AA. For example, the integrin β 2 hypomorph targeted mutation (*Itgb2^{tm1Bay}*, formerly CD18 targeted mutation) on the mouse PL/J congenic background, like the HLA-B27 rat, has features suggestive of both AA and psoriasis [154]. Like the DEBR rat and the C3H/HeJ mouse models for spontaneous AA (see below), these *Itgb2^{tm1Bay}* hypomorph mice regrow hair when monoclonal antibodies against CD4+ and CD8+ lymphocytes were injected, suggesting a similar inflammatory mechanism of hair loss in all three models. However, mapping phenotype modifier genes in the *Itgb2^{tm1Bay}* hypomorph congenic stock has progressed slowly.

Our investigations have identified 8 different strains that have an AA-like disease (table 2). One congenic strain, BALB.2R-*H2^{h2}*/Lil, has nail defects and may prove to be a model for human AA subtype in which patients have nail abnormalities [80]. Graft induction tests with the A/J mice were successful [unpubl. data]. However, these A/J mice may have a distinct AA subtype or clinical disease as there was a significant delay in time to over 25 weeks for AA to be clinically detected, compared to 10 weeks with the C3H/HeJ graft-induced model (see below).

Sexual Dichotomy in C3H/HeJ AA

In humans with autoimmune disorders, it has frequently been observed that females are disproportionately affected. In the C3H/HeJ mice with an AA-like hair loss, we observed that female mice develop AA earlier in age and more severely than males [59]. To investigate the roles of sex steroid hormones in the mouse AA-like disease, we neutered mice, both males and females, allowed them to heal, and then induced AA with skin grafts. This removal of gonadal hormones resulted in a significant delay in onset of AA in skin graft recipient mice [155]. These data implied that the delay of onset of anagen stage follicles was due to estrogen effects on hair follicle cycling. Dihydrotestosterone supplementation also inhibited onset perhaps due to a reduction in immune system activity. These results initially appeared to contradict the work of Oh and Smart [156], as suggested by Stenn et al. [157]. However, a subsequent paper [158] indicated that topical pharmacological application of estradiol yielded opposite effects on hair follicle cycling than systemic use, thus providing a rational basis for our results. A recent review from Ralf Paus' laboratory indicates that local and systemic effects of estrogen on mammalian hair follicle do not simply occur due to the presence of high-affinity estrogen receptors but also by modifying transcription of genes with estrogen response elements. 17- β -estradiol (E2) also modifies androgen metabolism within distinct parts of the hair follicle and sebaceous glands that have prominent aromatase activity. Aromatase is the key enzyme for androgen conversion to E2 and serves as an estrogen source and target of its action [159].

Humoral Immune System in Mouse AA

If autoantibodies to anagen follicles are a primary or secondary initiating event, what follicular antigens are the target(s)? Basement membrane proteins are the targets in autoimmune subepidermal bullous dermatoses, so it is reasonable that antigenic epitopes in follicular keratinocytes or melanocytes could be targeted in AA. Also, degradation of epidermal and follicular keratins normally occurs to prevent accumulation, such as might occur in cutaneous amyloidosis, but these keratins are also easy targets for a defective autoimmune response to the epitopes so generated. To examine the hypothesis that hair keratins play a primary role as the initiating targets in the mouse AA model, we collected sera to detect autoantibody production. Sera were collected on a monthly basis for over 12 months from 105 clinically normal female C3H mice to correlate onset of AA with the development of specific antibodies directed against anagen hair follicle proteins, specifically hair keratins. Twenty-three (22%) mice developed AA within the study period with initial onset of hair loss ranging from 4 to 12 months. Hair follicle antigens were recognized by antibodies present in sera from these C3H/HeJ mice born to AA-affected mothers. The heterogeneous

nature of the anti-hair follicle antibody responses, even in mice that did not develop AA within the study period, suggests that the development of anti-hair follicle antibodies alone may not be sufficient for the induction of AA-like hair loss [160]. Subsequent gene expression analysis on skin from graft-induced or spontaneous AA mice showed a late, nonspecific elevation of numerous immunoglobulin light chain transcripts [81]. From these studies we concluded that the humoral response to hair keratins is a secondary response to hair follicle root sheath disruption by infiltrating lymphocytes.

An alternate hypothesis is that C3H/HeJ mice and human AA patients in general have a fundamental abnormality in antibody production. This hypothesis was suggested by the observations that C3H/HeJ mice and human patients may both have concurrent AA and inflammatory bowel disease [161–163]. Both diseases appear to have an underlying dysregulation of the humoral immune system associated with anagen hair follicles (AA) [75] or endogenous bacterial flora (inflammatory bowel disease) [164]. Using intercrosses between C3H/HeJ and C57BL/6J (B6) mice, serum IgG and fecal (secretory) IgA levels directed against *Escherichia coli* cell wall antigens and anagen follicles were determined and used as quantitative traits. We identified three loci for fecal IgA levels, one of which mapped near the locus identified initially for mouse AA on mouse chromosome 6 (LOD score 4.9). Although intriguing, no correlation with IgG or IgA autoantibodies and anagen follicles was identified in the same sera by indirect immunofluorescence on frozen skin sections. Further studies indicated no correlation with this locus and AA (see below).

Cell-Mediated Immune System in Mouse AA

Is mouse and human AA due to a cell-mediated autoimmune response in an organ-specific manner? The majority of organ-specific autoimmune diseases are predominantly mediated by T lymphocytes and, if AA is an autoimmune disease, the disease mechanism might also be controlled by lymphocytes. The diagnostic histologic feature of AA is a marked lymphocytic infiltrate in and around anagen stage hair follicles. Therapeutic downregulation of this lymphocyte activation response often resolves the disease, further supporting a cell-mediated immunity basis to AA development. The laboratory mouse, with the wide assortment of immunologic reagents and genetic tools available, is an ideal model to test hypotheses on the mechanisms of AA.

Skin Graft Induction AA Model

What factors regulate the induction of AA? Are the lymphocytes activated by unique epitopes found only in the hair follicles of C3H/HeJ mice in this model or are the immunological mechanisms regulating tolerance defective, allowing normally suppressed lymphocytes to be persistently and clonally

activated? Gilhar et al. [165] showed, using a human skin xenograft model, that primed lymphocytes induce localized AA. In this model, activated lymphocytes are clearly the initiators of AA. Our mouse studies also show the ability of lymphocytes from AA-affected mice in vivo to induce localized AA and the AA subsequently become generalized (see below). The limitations of the human skin xenograft model and the long delay and variability in induction of spontaneous AA in C3H/HeJ made it imperative that a more efficient model be developed.

How could the C3H/HeJ AA model be made more useful? A skin graft model to transfer AA from spontaneous AA-affected C3H/HeJ mice onto immunocompetent, normal haired C3H mice was developed [135]. Within 10 weeks of grafting AA-affected skin onto immunocompetent C3H wild-type mice, ventral and dorsal patchy AA was induced that progressed to diffuse AA by 25 weeks after surgery [135]. The AA induction by the skin grafts was examined and found to have no infectious disease basis [135]. The initial studies determined that for this skin graft model to be reproducible, C3H/HeJ mice recipients had to be at least 3 months old for the full-thickness skin grafts from C3H/HeJ mice with AA to induce AA in the normal haired C3H/HeJ mice. Grafts onto severe combined immunodeficiency mice (*Prkdc^{scid}*) did not develop generalized AA and the alopecic graft sites regrew white hair, indicating lymphocytic immune cell activation was necessary to perpetuate the AA and transfer the disease from the graft to the graft recipient. Once this C3H/HeJ skin graft transfer of AA from affected C3H/HeJ to unaffected littermates was well characterized, studies were expanded to use this model to dissect which specific cells or processes are critical in the pathogenesis of AA [81, 166].

Evolution of AA in the C3H/HeJ AA Skin Graft Induction Model

If AA can be serially passed in the C3H/HeJ graft induction model, what histological, immunological and RNA changes could be detected to explain the evolution of AA? Mice received grafts of AA-affected skin and were necropsied at consecutive two-week intervals after surgery. Histopathology, transmission electron microscopy and indirect immunofluorescence studies were done on the graft site and at distant sites to follow progression of AA based on types of infiltrating cells. RNA was extracted from skin for Affymetrix Genechip® and quantitative PCR analyses [81]. Histologically, the surgical sites were completely healed by 2 weeks after surgery. There was scarring within the graft itself, enabling easy identification of the borders with normal host skin. These junctions were associated with wound-mediated induction of a narrow but consistent area of anagen stage hair follicles in the host skin. Beyond this border, on the host skin side, the follicles were predominantly in telogen. Those mice that received normal donor skin grafts had little to no inflammation in or around the

anagen hair follicles in the graft itself, the normal host skin adjacent to the graft, or distant sites at any time after the graft surgery. In contrast, there was a mixed inflammatory cell infiltrate in and around anagen follicles of both donor and recipient skin from those receiving grafts from AA mice. This dichotomy continued in successive weeks after grafting. Those mice receiving skin grafts from mice with AA developed extensive peri- and interfollicular inflammation, primarily lymphocytic, focused on anagen stage follicles located in extensive areas around the graft and in distant sites by 8 weeks after engraftment. This feature persisted at 10 and 12 weeks after grafting. Hair follicles from the AA graft recipients in late anagen and early catagen had a prominent inflammatory component, but also exhibited marked follicular dystrophy in the region of the matrix above the bulb that resulted in disintegration of the hair fiber [167].

Grafts from AA mice onto normal recipients had CD8+ T cells (by indirect immunofluorescence) within the graft on day 0. These cells localized immediately adjacent to the graft site by 4 weeks after surgery. By 8 weeks, intense CD8+ cell infiltrates were located in a perifollicular pattern in host anagen stage hair follicles. CD8+ T cell infiltration in the dermis was more diffuse and less focused on hair follicles in the graft recipient mice at this time point than typically observed in spontaneous, chronic AA-affected mice. By 10 weeks after surgery, intrafollicular penetration by individual CD8+ T cells was apparent, typical of the spontaneous form of this disease in mice. Similar features were observed at 12 weeks after surgery. The hair follicle root sheaths expressed ICAM-1 four weeks after graft surgery. The intensity of ICAM-1 expression varied with individual hair follicles and persisted until overt hair loss [167].

Transmission electron microscopy of samples taken in the immediate AA graft site revealed isolated lymphocytes in a perifollicular location by 6 weeks after grafting. Disorganization of hair follicle root sheaths was evident by 8 weeks after grafting. Lymphocyte, macrophage and polymorphonuclear cell infiltration was prominent in peri- and intrafollicular locations (within the outer and inner root sheaths) 8 weeks after grafting. Extensive hair follicle dystrophy was apparent by 10 weeks after grafting. Controls (normal skin grafted onto normal, histocompatible recipients) exhibited no apparent hair follicle inflammation or disorganization at any time point after surgery [167].

Studies evaluating the development of AA in the induced skin graft model using flow cytometry have shown changes in cytokine expression, as AA is first induced and then progresses in the mice. Two weeks after transplantation of AA-affected skin, expression of IL-2, IL-4, IL-6 and IL-12 in the skin is significantly elevated compared with normal haired mice. Expression of TNF- α , IL-10 and IFN- γ is also somewhat upregulated but their levels are not as high as in chronic AA-affected mice. However, by 12 weeks after transplanting

AA-affected skin, the cytokine expression pattern resembles one seen mice with chronic AA. While the initial stages of AA development are associated with bias more towards Th1 cytokine expression, the cytokine expression profile in chronic AA-affected mouse skin does not define an unequivocal Th1 versus Th2 cytokine dominating state. The monocyte-derived inflammatory cytokines TNF- α and IL-6 are expressed at an elevated level and increased expression of Th1 cytokines IL-12 and IFN- γ as well as Th2 cytokines IL-4 and IL-10 are observed. It seems that both Th1 and Th2 mechanisms may be active in chronic mouse AA [166].

CD4+/CD25+ cells are known to be important for lymphocyte homeostasis [168]. It is also known that autoimmune reactions in the absence of CD40 are characterized by a significant decrease in the number of CD4+/CD25+ regulatory cells [169]. As AA progresses in the skin graft model, a low level of CD40+ and CD4+/CD25+ cells can be identified in the skin and lymph nodes of AA-affected mice [170]. This may indicate AA-affected mice are unable to properly regulate peripheral immune activity and this may be significant for AA susceptibility. Taken together, the AA characterization studies support the concept of AA as an autoimmune disease and have elucidated possible mechanisms of inflammatory cell activation, regulation, migration and hair follicle activity modulation.

Mechanisms Involved in the Graft Induction Mouse Model for AA

What factors regulate this induction of AA in the skin graft transfer model? Are the lymphocytes activated by unique epitopes found only in the hair follicles of C3H/HeJ or are the immunological mechanisms regulating tolerance defective, allowing lymphocytes normally suppressed to be persistently and clonally activated? Gilhar et al. [165], using a human skin xenograft model, showed that primed lymphocytes induce localized AA. Although activated lymphocytes are clearly the initiators of rodent AA, our mouse studies also show the ability of lymphocytes from AA-affected mice *in vivo* to induce localized AA that subsequently becomes generalized.

Our current hypothesis to explain the induction of AA presumes that AA is a complex polygenic trait influenced by epigenetic events, primarily environmental. An antigenic epitope, presumed to be endogenous, such as hair-specific hard acidic keratins (trichohyalin), or exogenous infectious or chemical agents (identity unknown), are recognized by APCs. These APCs have CD80 (formerly B7.1) and CD86 (formerly B7.2) lymphocyte costimulatory molecules on their surface. The APCs that recognize the antigenic epitope then migrate to regional lymph nodes that are the most efficient centers for AA induction. A complex of CD80 and CD86 ligands and CD28 T cell surface receptors in the presence of antigen signals promotes T cell proliferation, enhances cytokine production and

induces *Bc-x* that promotes T cell survival. T cells with CD44var.10 surface receptors then migrate to the skin, homing on hair follicles in the anagen stage of the hair cycle, thus initiating the first stages of clinical AA [171].

Subsequent studies confirmed that CD44var.10 T cell surface receptors and other CD44 variants are upregulated early in the evolution of AA and later downregulated as the disease progresses to a chronic disease state. It seems that expression of CD44 receptors may be important for the induction of AA, but they are relatively less involved in maintaining AA [166]. Functional studies using the skin graft model support this view. Onset of AA is partially or completely inhibited in the skin graft model with administration of anti-CD44var.10 monoclonal antibodies at the time of skin grafting to deplete CD44var.10-expressing cells [171]. The CD18 hypomorph model (*Itgb2^{tm1Bay}*) provides additional support for the CD44 blocking studies in the AA mouse model. Although the CD18 hypomorph targeted mutant mouse develops a psoriasis/AA-like inflammatory skin disease [154], a true CD18 null mutant (*Itgb^{tm2Bay}*) develops only spontaneous skin ulcers. CD18^{-/-} mice cannot mount a contact sensitivity response, but CD18^{-/-} cells can be primed to antigens, although the primed T cells cannot home to the skin associated with accumulation of CD3-CD44^{high} in lymph nodes [172].

How are the antigenic epitopes that induce a humoral response exposed to APCs? Once there is a disruption of the hair follicle integrity by a cellular immune response, the previously immunologically privileged sites are exposed, thereby inciting a secondary humoral response. These secondary humoral immunological responses may play a role in the perpetuation of AA. There is strong upregulation of TNF- α (*Tnfa*) mRNA transcripts during the early phase of wound healing in normal mice, with *Tnfa* levels at 24 h that were 30-fold greater than those observed in the days just prior to rejection of allografts [173]. *Tnfa* is a pleiotropic molecule important in promoting normal wound healing [174–176] in addition to its ability to induce necrosis likely to be involved in allograft rejection. Innate immune response functions attributed to *Tnfa* include (1) a stimulus for migration of APCs from the epidermis to draining lymph nodes [177, 178], (2) induction of the expression of vascular cell adhesion molecule 1 (VCAM-1), ICAM-1 and SELE [179–182], and (3) induction of the expression of MHC class I molecules [182]. This process is presumably important in the initiating events of this graft induction model and possibly also its role in the genetic aspects of AA in humans [183].

Transcript analysis using Affymetrix Genechips and selected quantitative RT-PCR were done on RNA from skin of mice with and without AA as well as sequentially after grafting AA-affected skin or normal skin onto C3H/HeJ mice. Cluster analyses revealed various patterns of altered gene expression. Analyses focused on early upregulation of genes involved in lymphocyte activation and

late upregulation of genes coding for variable chains of immunoglobulins [81]. Wide-scale upregulation of transcripts coding for a large number of immunoglobulins as well as melanin-associated proteins was a late event in this model [81].

What direct evidence is there that a cellular immune response is the primary inciting event in AA? Strong support for the T cell activation via a costimulation mechanism comes from the C3H/HeJ inducible AA graft model. AA full-thickness grafts onto C3H/HeJ haired mice induced typical AA within 10 weeks after surgery at multiple sites distant from the graft [135]. Onset of AA is partially or completely inhibited in this model after administration of anti-CD80/CD86 monoclonal antibodies or Ctl4-Ig [81] to block the T cell activation via costimulatory pathways. Blocking of T cell activation in regional lymph nodes was likely the result of inactivation of CD80/CD86 on APCs or downregulating activation by competing for binding sites. Blocking of this pathway with the fusion protein Ctl4-Ig has potential therapeutic benefits in other diseases as well [184].

Are there other immunological signaling pathways that may regulate important events in the pathogenesis of AA? Another mechanism potentially operative in AA as a T cell-mediated autoimmune disease of hair follicles is Fas and Fas ligand (FasL). Fas is expressed on hair follicles and FasL on perifollicular infiltrates. If the Fas-FasL pathway is of pathogenic significance in AA, then mice with mutations in this pathways should either develop AA or be resistant to AA transferred in the graft induction model. Fas-deficient (C3H/HeJ-*Fas^{gld}*), FasL-deficient (C3.MRL-*FasL^{lpr}*) and normal histocompatible C3H/HeJ mice were grafted with skin from AA-affected C3H/HeJ mice. All normal wild-type C3H/HeJ mice developed AA, while no Fas-deficient mice showed hair loss and 2 of 7 FasL-deficient mice developed only transitory, limited AA. Moreover, doing the reverse experiment in which skin from wild-type mice, C3H/HeJ-*Fas^{gld}* and C3.MRL-*FasL^{lpr}* mice was grafted onto C3H/HeJ mice with extensive AA showed that the normal wild-type mouse skin developed hair loss, whereas Fas-deficient and FasL-deficient skin grafts did not develop AA. TUNEL and immunofluorescence studies showed an increased number of apoptotic cells and expression of Fas on hair follicles as well as expression of FasL on cells of the perifollicular infiltrate in C3H/HeJ mice with AA. In contrast, in Fas-deficient and FasL-deficient mice, apoptotic cells were virtually absent in and around hair follicles. Collectively, these data indicate that the Fas-FasL pathway is important in the pathogenesis of mouse AA [185] and presumably also in human AA.

How similar are these data when comparing the pathogenesis of mouse AA to human AA? Similar gene expression studies were done on skin collected from human AT patients compared with normal age-, sex- and biopsy site-matched

people. Both similarities and differences between the two species were identified, indicating a need to correlate the stage of disease with gene expression data as well as to carefully identify the subtypes of human AA. The mouse AA-like disease appears to remain relatively active even when it has been ongoing for several months in contrast to human AT patients, in which it is difficult to show continued T cell activation and perifollicular infiltrates [81]. Regardless, microarray technology has defined several inflammatory mechanisms involved in mouse and human AA that could be targets for new treatment interventions in humans with AA. New generation array technology is expanding these discoveries.

Cell Transfer and Depletion Studies

If AA is a cell-mediated autoimmune skin disease as commonly believed, and not primarily a humoral-mediated autoimmunity phenomenon, inactivating or depleting effector cells should affect the onset, severity and/or duration of AA in susceptible mice. The purported effector cells, CD4+ and CD8+ T lymphocytes based on immunohistochemical studies, were depleted from affected C3H/HeJ mice using monoclonal antibodies [81]. Hair regrowth was observed in monoclonal antibody-treated AA mice when either CD4+ or CD8+ lymphocytes were depleted although hair regrowth was not complete. Subsequently, AA returned in the monoclonal antibody-treated mice when CD4+ or CD8+ cells increased after stopping monoclonal antibody treatment. These data supported the hypothesis that systemic removal of either CD4+ or CD8+ lymphocytes from bone marrow, spleen and/or draining lymph nodes would diminish or resolve AA in affected mice [81].

Cells from bone marrow, spleen or draining subcutaneous lymph nodes were removed from affected mice and injected into unaffected littermates. Transfer of putatively activated cells of the immune system from AA-affected mice to immunocompetent mice yielded various degrees of efficiency of AA induction. Bone marrow did not transfer AA, while cells from spleens and draining lymph nodes induced AA in 30 and 70% of recipients, respectively [81]. These studies indicated leukocytes present in skin-associated lymph nodes are involved in the induction of AA. Skin-draining lymph nodes are the anatomical site of the highest activation state of lymphocytes consistent with lymphocytes and other undefined cells from the lymph nodes being the most efficient in inducing AA.

Further studies have subsequently evaluated specific draining lymph node-derived cell subsets for their AA-inducing and AA-regulating capabilities. Purified cell subsets from skin-draining lymph nodes of AA-affected mice were isolated using magnetic bead-conjugated antibodies and injected subcutaneously into normal haired mice. CD8+ cell-injected mice exhibited hair loss

limited to the injection site. In contrast, some CD4⁺ and CD4⁺/CD25[−] cell-injected mice developed multiple patches of AA beyond the site of injection, while a combined injection of CD8⁺ and CD4⁺/CD25[−] cells increased the frequency of AA development in recipient mice. The lymph nodes and skin-infiltrating leukocytes were examined by flow cytometry after cell injection. There was a strong upregulation of IL-6, TNF- α , IL-12 and IFN- γ expression in mice developing localized or extensive AA. Increase in costimulatory ligand CD80, plus increased leukocyte apoptosis resistance and reduced Fas, FasL and CD120b expression were associated with successful AA induction. The results were consistent with CD8⁺ cells as the primary effectors of hair loss phenotype, while extensive disease expression is apparently determined by CD4⁺/CD25[−] cells. In combination, CD4⁺/CD25[−] and CD8⁺ cells acted synergistically, suggesting CD4⁺/CD25[−] cells may induce hair loss primarily via their activation of autoreactive CD8⁺ cells [186].

Identity of Reactive Epitopes: Keratinocytes and Melanocytes in Human and Mouse AA

Where are the reactive epitopes that are exposed when immune privilege is no longer present in inflamed hair follicles? A persistent hypothesis is that melanocytes or melanogenesis-related proteins (MRPs) are the target(s) for the immune system in AA. This is mainly due to the clinical observation that white hair often regrows in human patients with AA and these hairs are resistant to future recurrences. We observed white hair regrowth of AA grafts onto immunodeficient mice and in some of the spontaneous cases in C3HB6F1 and F2 hybrids. To address this hypothesis, we induced formation of white hair using freeze branding in the AA graft model. In 13 of 14 AA grafted mice, white hairs were not protected from onset of AA. Our interpretation is that white hairs are a result of injury and a secondary effect on hair pigmentation [155]. This conclusion might be further supported by the observation of AA-like phenotypes in several albino strains [80]. Late upregulation of many MRP genes in our graft model also suggests this may be a secondary event in the pathogenesis of AA [65]. The xenograft model for relapsing AA by Gilhar et al. [85] also showed no single melanocyte antigen is generally associated with AA.

Is there any evidence that hard keratinocytes such as trichohyalin or cellular proteins residing in the hair bulge may be the targets for immune damage? There is minimal evidence to identify reactive epitopes in keratinocytes or other nonpigmented cells in human AA [12, 71–75, 187, 188]. In humans, the most characteristic location of peri- and intrafollicular lymphocytic inflammation is in the hair bulbar region and not around the hair bulge (fig. 2). This localization is characteristic, but does not aid in identifying a specific protein

as seen in bullous autoimmune diseases in humans. In mice, the lymphocyte inflammation is not as totally focused on the hair bulb, but is still not up higher or inducing basal cell damage as seen in lupus erythematosus or lichen planopilaris, both of which are scarring alopecias. Inflammation in AA in the acute and subacute stages does not clinically induce scarring and often the process may spontaneously remit; however, changes in serological titers of specific proteins have not consistently identified the targets of the autoantibodies or when identified, they are also present in nonaffected human subjects [12, 71–75, 187, 188].

Genetics of Mouse AA

What genetic evidence documents that the C3H/HeJ AA model is useful to study human AA? More specifically, is there evidence that the dysregulated mouse and human genes and protein signaling pathways are the same or at least correspond? The initial approach was to cross female and male C3H/HeJ mice with AA or pairs in which only one mouse had the disease. Progeny were aged and evaluated for onset of disease, but it was quickly obvious that mouse AA in this model was not due to a simple, single, autosomal and recessive mutation. Rather, some of the F1 generation developed disease suggesting that one or more loci had a dominant or semidominant effect. The low frequency of affected mice in litters suggested that up to four independent, unlinked loci might be involved in the C3H/HeJ model. Their progeny were then backcrossed with C57BL6J mice with the goal of identifying dominant genes. A highly significant genomic interval containing AA susceptibility genes was found on mouse chromosome 17 and a marginally significant interval on mouse chromosome 9. These intervals were subsequently assigned the designations *Alaa1* and *Alaa2*, respectively, by the International Mouse Nomenclature Committee. *Alaa1* includes H2 (human HLA ortholog), TNF- α (*Tnfa*) and lymphotoxin α and β (*Ltfa*, *Lfib*). *Alaa2* contains genes that code for proteins involved with T cell function (*Cd3*, *Thy1* and regulatory T cell molecule, *Ctram*), cytokines that regulate inflammation (*Il1*, *Ora*) and neural cell adhesion molecule (*Ncam1*). Many human gene association studies have focused on HLA linkage to AA, making the mouse *Alaa1* interval containing the homologous group of genes an important focus, and that strongly supports the value of the C3H/HeJ model for human AA. By refining the statistics for *Alaa1* and *Alaa2*, significant intervals on mouse chromosome 8 (*Alaa 3*) and mouse chromosome 15 (*Alaa4*) were identified. These intervals contain a variety of immunoregulatory genes that are candidates for analysis of genes responsible for susceptibility to AA. Other human studies identified genes not found in this linkage study, but these human transcription factors are directly regulated by genes within *Alaa1*. These results indicate the necessity

of integrating both gene association and genome-wide linkage studies in both mice and humans to understand the complex nature of AA and other polygenic diseases [189, 190].

How has the skin graft transfer model using C3H/HeJ mice led to a better understanding of AA? Can this model be further exploited to take advantage of the dramatic advances in gene expression technology made in the last 5 years? With pilot funding from the National Alopecia Areata Foundation to take advantage of recent technical advances, we performed full-thickness skin grafts from mice with AA or normal mice onto young normal C3H/HeJ mice and collected skin for RNA extraction, histopathology, immunofluorescence and other assays at 5, 10, 15 and 20 weeks after surgery, using age- and gender-matched mice with spontaneous onset of AA or unaffected mice by clinical and histological criteria. Samples were tested using the Affymetrix Genechip Mouse Genome 430 2.0 Array. Data generated were analyzed with the help of B. King (TJL Computational Biology Shared Service), using the Ingenuity[®] Network Analysis Systems (<http://analysis.ingenuity.com/pa>) software. We found that the genes and gene networks dysregulated in our earlier Affymetrix studies were similar, except that far more information is now accessible, especially with the newly available software to analyze these results [Sundberg et al., unpubl. data]. Our analyses of these data sets are not complete but we have intriguing findings that provide a logical plan for systematic drug screening approaches for AA.

Mouse AA Models to Test Role(s) of Signaling Pathways in Pathogenesis of AA

How else can the skin graft induction model of AA be used productively? The AA graft model is an ideal way to produce large numbers of readily available, reproducible and predictably induced AA mice for therapeutic screening studies. Our initial studies showed that spontaneously occurring AA in C3H/HeJ mice could be successfully treated by using intralesional corticosteroid injections similar to the response in patchy, human AA patients [59]. A number of other compounds have been used with various degrees of success to treat human AA, including immunomodulatory agents such as diphencyprone, anthralin and squaric acid dibutyl ester. These compounds were found to be effective in the spontaneous, chronic AA mouse model as well as in the graft induction model [139, 142, 191]. Experimental agents that affect different pathways in the immune system in mice and humans have already been tested [140, 170]. Future plans with these established and new collaborators are to use this AA model, particularly the graft-induced AA model, to explore the mechanisms whereby hair regrows following treatment with agents commonly used to treat human AA.

Acknowledgements

The authors thank K.A. Silva for her technical assistance. This work was supported by grants from the National Alopecia Areata Foundation (J.P.S., L.E.K., K.J.M.), the National Institutes of Health (AR43801, RR173 and CA34196 to J.P.S.; 5P30AR041943-13 to L.E.K.), the Council for Nail Disorders (J.P.S.) and the Dermatology Foundation/Glaxo Dermatology Fellowship (K.J.M.).

References

- 1 Muller SA, Winkelmann RK: Alopecia areata: an evaluation of 736 patients. *Arch Dermatol* 1963;88:290–297.
- 2 Safavi KH, Muller SA, Suman VJ, Moshell AN, Melton LJ: Incidence of alopecia areata in Olmstead County, Minnesota 1975–1989. *Mayo Clin Proc* 1995;70:628–633.
- 3 Wang S-J, Shohat T, Vadheim C, Shellow W, Edwards J, Rotter JJ: Increased risk for type I (insulin-dependent) diabetes in relatives of patients with alopecia areata (AA). *Am J Med Genet* 1994;51:234–239.
- 4 Cunliffe WJ, Hall R, Stevenson CJ, Weightman D: Alopecia areata, thyroid disease and autoimmunity. *Br J Dermatol* 1969;81:877–881.
- 5 Milgraum SS, Mitchell AJ, Bacon GE, Rasmussen JE: Alopecia areata, endocrine function, and autoantibodies in patients 16 years of age or younger. *J Am Acad Dermatol* 1987;17:57–61.
- 6 Shellow WV, Edwards JE, Koo JY: Profile of alopecia areata: a questionnaire analysis of patient and family. *Int J Dermatol* 1992;31:186–189.
- 7 Puavilai S, Puavilai G, Charuwichitratana S, Sakuntabhai A, Sriprachya-Anunt S: Prevalence of thyroid diseases in patients with alopecia areata. *Int J Dermatol* 1994;33:632–633.
- 8 Kurtev A, Iliev E: Thyroid autoimmunity in children and adolescents with alopecia areata. *Int J Dermatol* 2005;44:457–461.
- 9 Liu JB, Li M, Yang S, Gui JP, Wang HY, Du WH, Zhao XY, Ren YQ, Zhu YG, Zhang XJ: Clinical profiles of vitiligo in China: an analysis of 3,742 patients. *Clin Exp Dermatol* 2005;30:327–331.
- 10 Goh C, Finkel M, Christos PJ, Sinha AA: Profile of 513 patients with alopecia areata: associations of disease subtypes with atopy, autoimmune disease and positive family history. *J Eur Acad Dermatol Venereol* 2006;20:1055–1060.
- 11 Schallreuter KU, Lemke R, Brandt O, Schwartz R, Westhofen M, Montz R, Berger J: Vitiligo and other diseases: coexistence or true association? Hamburg study of 321 patients. *Dermatology* 1994;188:269–275.
- 12 Sperling LC: Alopecias; in Bologna JL, Jorizzo JL, Rapini P: *Dermatology*. Ed 2, Mosby Elsevier, 2008, pp 998–1007.
- 13 Ikeda T: A new classification of alopecia areata. *Dermatologica* 1965;131:421–445.
- 14 Penders AJ: Alopecia areata and atopy. *Dermatologica* 1968;136:395–399.
- 15 de Waard-van der Spek FB, Oranje AP, de Raeymaecker DM, Peereboom-Wynia JD: Juvenile versus maturity-onset alopecia areata – a comparative retrospective clinical study. *Clin Exp Dermatol* 1989;14:429–433.
- 16 Weise K, Kretzschmar L, John SM, Hamm H: Topical immunotherapy in alopecia areata: anamnestic and clinical criteria of prognostic significance. *Dermatology* 1996;192:129–133.
- 17 Sabouraud R: Sur les origines de la pelade. *Ann Dermatol Syphiligr (Paris)* 1896;3:253–277.
- 18 Barber H: Alopecia areata. *Br J Dermatol Syphilis* 1921;33:1–14.
- 19 Bowen J: Two epidemics of alopecia areata in an asylum for girls. *J Cutaneous Genito-Urin Dis* 1899;17:399–404.
- 20 Fox TC: On a small epidemic of an areate alopecia. *Br J Dermatol* 1913;25:51–56.
- 21 Davis H: Epidemic alopecia areata. *Br J Dermatol* 1914;26:207–210.

- 22 Roxburgh AC: The aetiology of alopecia areata and its relationship to vitiligo and scleroderma. *Br J Dermatol Syphilis* 1929;41:351–363.
- 23 Ormsby OS: Diseases of the skin, ed 7. London, Henry Kimpton, 1948.
- 24 Ikeda T: Produced alopecia areata based on the focal infection theory and mental motion theory. *Dermatologica* 1967;134:1–11.
- 25 Skinner RB, Light WH, Bale GF, Rosenberg EW, Leonardi C: Alopecia areata and presence of cytomegalovirus DNA. *J Am Med Assoc* 1995;273:1419–1420.
- 26 Skinner RB, Light WH, Leonardi C, Bale GF, Rosenberg EW: A molecular approach to alopecia areata. *J Invest Dermatol* 1995;104(suppl):3S–4S.
- 27 Hernandez MJG, Torres MJ, Palomares JC, Rodriguez-Pichardo A, Aznar J, Camacho F: No evidence of cytomegalovirus DNA in alopecia areata. *J Invest Dermatol* 1998;110:185.
- 28 Tosti A, Placa ML, Placucci, Gentilomi G, Venturoli S, Zerbini M, Musiani M: No correlation between cytomegalovirus and alopecia areata. *J Invest Dermatol* 1996;107:443.
- 29 Jackow C, Puffer N, Hordinsky M, Nelson J, Tarrand J, Duvic M: Alopecia areata and cytomegalovirus infection in twins: genes versus environment? *J Am Acad Dermatol* 1998;38:418–425.
- 30 Offidani A, Amerio P, Bernardini ML, Fliciani C, Bossi G: Role of cytomegalovirus replication in alopecia areata pathogenesis. *J Cutan Med Surg* 2000;4:63–65.
- 31 Freshwater D: Alopecia areata. *Practitioner* 1915;95:796–806.
- 32 Joseph M: Experimentelle Untersuchungen über die Ätiologie der Alopecia areata. *Monatsh Prakt Dermatol* 1886;5:483–489.
- 33 Slominski A: Neuroendocrine system of the skin. *Dermatology* 2005;211:199–208.
- 34 Kingsbury J: Three cases of alopecia areata associated with ametropia. *J Cut Dis* 1909;27:211.
- 35 Hordinsky MK, Kennedy W, Wendelschafer-Crabb G, Lewis S: Structure and function of cutaneous nerves in alopecia areata. *J Invest Dermatol* 1995;104(suppl):28S–29S.
- 36 Hordinsky MK, Ericson ME: Relationship between follicular nerve supply and alopecia. *Dermatol Clin* 1996;14:651–660.
- 37 Hordinsky MK: Alopecia areata; in Olson EA: Disorders of Hair Growth. New York, McGraw-Hill, 1994, pp 195–222.
- 38 Adamson HG: Modern Views Upon the Significance of Skin Eruptions. London, John Bale & Sons, 1912.
- 39 Brown WH: The aetiology of alopecia areata and its relationship to vitiligo and possibly scleroderma. *Br J Dermatol Syphilis* 1929;41:299–323.
- 40 Dixon WE: Thallium. *Proc R Soc Med* 1927;20:1197–1200.
- 41 Meachen GN, Provis FL: Case of alopecia areata et totalis. *Br J Dermatol* 1912;24:272–274.
- 42 Sabouraud R: Nouvelles recherches sur l'étiologie de la pelade (pelade et ménopause). *Ann Dermatol Syphiligr (Paris)* 1913;5:88–97.
- 43 Sabouraud R: Pelade et goitre exophthalmique. *Ann Dermatol Syphiligr (Paris)* 1913;5:140–148.
- 44 Levy-Franckel A, Juster E: La pelade. *J Med Paris* 1925;44:631–635.
- 45 Sharma VK, Sialy R, Kumar B, Gupta S: Evaluation of thyroid function in north Indians with alopecia areata: response to intravenous injection of 100 micrograms thyrotropin releasing hormone (TRH). *J Dermatol* 1999;26:339–342.
- 46 Collins SM, Dominguez M, Ilmarinen T, Costigan C, Irvine AD: Dermatological manifestations of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome. *Br J Dermatol* 2006;154:1088–1093.
- 47 Giovannini S: Recherches sur l'histologie pathologique de la pelade. *Ann Dermatol Syphiligr (Paris)* 1891;3:921–957.
- 48 Van Scott EJ: Morphologic changes in pilosebaceous units and anagen hairs in alopecia areata. *J Invest Dermatol* 1958;31:35–43.
- 49 Paus R, Slominski A, Czarnetzki BM: Is alopecia areata an autoimmune-response against melanogenesis-related proteins exposed by abnormal MHC class I expression in the anagen hair bulb? *Yale J Biol Med* 1993;66:541–554.
- 50 McDonagh AJ, Messenger AG: The aetiology and pathogenesis of alopecia areata. *J Dermatol Sci* 1994;7(suppl):S125–S135.
- 51 McDonagh AJ, Messenger AG: The pathogenesis of alopecia areata. *Dermatol Clin* 1996;14:661–670.

- 52 Bystryn J-C, Tamesis J: Immunologic aspects of hair loss. *J Invest Dermatol* 1991;96:88S–89S.
- 53 McElwee KJ, Tobin DJ, King LE, Sundberg JP: Alopecia areata: an autoimmune disease? *Exp Dermatol* 1999;8:371–379.
- 54 Hordinsky MK: Alopecia areata: pathophysiology and latest developments. *J Cutan Med Surg* 1999;3(suppl 3):S28–S30.
- 55 Randall VA: Is alopecia areata an autoimmune disease? *Lancet* 2001;358:1922–1924.
- 56 Perret C, Wiesner-Menzel L, Happle R: Immunohistochemical analysis of T-cell subsets in the peribulbar and intrabulbar infiltrates of alopecia areata. *Acta Derm Venereol* 1984;64:26–30.
- 57 Ranki A, Kianto U, Kanerva L, Tolvanen E, Johansson E: Immunohistochemical and electron microscopic characterization of the cellular infiltrate in alopecia (areata, totalis, and universalis). *J Invest Dermatol* 1984;83:7–11.
- 58 Zhang JG, Oliver RF: Immunohistochemical study of the development of the cellular infiltrate in the pelage follicles of the DEBR model for alopecia areata. *Br J Dermatol* 1994;130:405–414.
- 59 Sundberg JP, Cordy WR, King LE: Alopecia areata in aging C3H/HeJ mice. *J Invest Dermatol* 1994;102:847–856.
- 60 Wiesner-Menzel L, Happle R: Intrabulbar and peribulbar accumulation of dendritic OKT 6-positive cells in alopecia areata. *Arch Dermatol Res* 1984;276:333–334.
- 61 Paus R: Immunology of the hair follicle; in Bos JD: *Skin Immune System (SIS): Cutaneous Immunology and Clinical Immunodermatology*, ed 2. Boca Raton, CRC Press, 1997, pp 377–398.
- 62 Barker CF, Billingham RE: Immunologically privileged sites. *Adv Immunol* 1977;25:1–54.
- 63 Westgate GE, Craggs RI, Gibson WT: Immune privilege in hair growth. *J Invest Dermatol* 1991;97:417–420.
- 64 Messenger AG, Bleehen SS: Expression of HLA-DR by anagen hair follicles in alopecia areata. *J Invest Dermatol* 1985;85:569–572.
- 65 Khoury EL, Price VH, Greenspan JS: HLA-DR expression by hair follicle keratinocytes in alopecia areata: evidence that it is secondary to the lymphoid infiltration. *J Invest Dermatol* 1988;90:193–200.
- 66 Brocker E-B, Echternacht-Happle K, Hamm H, Happle R: Abnormal expression of class I and class II major histocompatibility antigens in alopecia areata: modulation by topical immunotherapy. *J Invest Dermatol* 1987;88:564–568.
- 67 Lu W, Shapiro J, Yu M, Barekatin A, Lo B, Finner A, McElwee K: Alopecia areata: pathogenesis and potential for therapy. *Expert Rev Mol Med* 2006;20:1–19.
- 68 McDonagh AJG, Snowden JA, Stierle C, Elliott K, Messenger AG: HLA and ICAM-1 expression in alopecia areata in vivo and in vitro: the role of cytokines. *Br J Dermatol* 1993;129:250–256.
- 69 Nickoloff BJ, Griffiths CEM: Aberrant intercellular adhesion molecule-1 (ICAM-1) expression by hair-follicle epithelial cells and endothelial leukocyte adhesion molecule-1 (ELAM-1) by vascular cells are important adhesion-molecule alterations in alopecia areata. *J Invest Dermatol* 1991;96:91S–92S.
- 70 Hoffmann R, Wenzel E, Huth A, van der Steen P, Schaufele M, Henninger H-P, Happle R: Cytokine mRNA levels in alopecia areata before and after treatment with the contact allergen diphenylcyclopropenone. *J Invest Dermatol* 1994;103:530–533.
- 71 Tobin DJ, Orentreich N, Fenton DA, Bystryn J-C: Antibodies to hair follicles in alopecia areata. *J Invest Dermatol* 1994;102:721–724.
- 72 Tobin DJ, Orentreich N, Bystryn J-C: Autoantibodies to hair follicles in normal individuals. *Arch Dermatol* 1994;130:395–396.
- 73 Tobin DJ: Alopecia areata is associated with antibodies to hair follicle-specific antigens located predominantly in the proliferative region of hair follicles; in van Neste D, Randall V: *Hair Research for the Next Millennium*. Elsevier, Amsterdam, 1996, pp 237–241.
- 74 Tobin DJ, Hann S-K, Song M-S, Bystryn J-C: Hair follicle structures targeted by antibodies in alopecia areata. *Arch Dermatol* 1997;133:57–61.
- 75 Tobin DJ, Sundberg JP, King LE, Boggess D, Bystryn J-C: Autoantibodies to hair follicles in C3H/HeJ mice with alopecia areata-like hair loss. *J Invest Dermatol* 1997;109:329–333.

- 76 McElwee KJ, Pickett P, Oliver RF: Hair follicle autoantibodies in DEBR rat sera. *J Invest Dermatol* 1995;104(suppl):34S–35S.
- 77 McElwee KJ, Pickett P, Oliver RF: The DEBR rat, alopecia areata and autoantibodies to the hair follicle. *Br J Dermatol* 1996;134:55–62.
- 78 McElwee KJ, Drummond S, Oliver RF: Hair follicle specific autoantibodies associated with alopecia areata in sera from the DEBR rat model and humans; in van Neste D, Randall V: *Hair Research for the Next Millennium*. Elsevier, Amsterdam, 1996, pp 253–257.
- 79 Tan RSH, Samman PD: Thymoma, acquired hypogammaglobulinemia, lichen planus, alopecia areata. *Proc R Soc Med* 1974;67:196–198.
- 80 McElwee K, Boggess D, Miller J, King L, Sundberg J: Spontaneous alopecia areata-like hair loss in one congenic and seven inbred laboratory mouse strains. *J Invest Dermatol Symp Proc* 1999;4:202–206.
- 81 Carroll J, McElwee KJ, King LE, Byrne MC, Sundberg JP: 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.
- 82 Grabar P: Autoantibodies and the physiological role of immunoglobulins. *Immunol Today* 1983;4:337–340.
- 83 Maran R, Dueymes M, Corre RL, Renaudineau Y, Shoenfeld Y, Youinou P: IgG subclasses of human autoantibodies. *Ann Med Intern* 1997;148:29–38.
- 84 El-Roiy A, Gross WL, Ludemann J, Isenberg DA, Shoenfeld Y: Preferential secretion of a common anti-DNA idiotype (16/6 Id) and anti-polynucleotide antibodies by normal mononuclear cells following stimulation with *Klebsiella pneumoniae*. *Immunol Lett* 1986;12:313–319.
- 85 Gilhar A, Landau M, Assy B, Shalaginov R, Serafimovich S, Kalish RS: Melanocyte-associated T cell epitopes can function as autoantigens for transfer of alopecia areata to human scalp explants on Prkdc^{scid} mice. *J Invest Dermatol* 2001;117:1357–1362.
- 86 Colombe BW, Price VH, Khoury EL, Garovoy MR, Lou CD: HLA class II antigen associations help to define two types of alopecia areata. *J Am Acad Dermatol* 1995;33:757–764.
- 87 van der Steen P, Traupe H, Happle R, Boezeman J, Sträter R, Hamm H: The genetic risk for alopecia areata in first degree relatives of severely affected patients: an estimate. *Acta Derm Venereol* 1992;72:373–375.
- 88 Green J, Sinclair RD: Genetics of alopecia areata. *Australas J Dermatol* 2000;41:213–318.
- 89 Barsky S, Gigli I: Alopecia areata in twins. *Arch Dermatol* 1961;83:224–225.
- 90 Bonjean M, Prime A, Avon P: Pelade chez deux jumeaux homozygotes (sic). *Lyon Med* 1968;219:1852–1853.
- 91 Cole GW, Herzlinger D: Alopecia universalis in identical twins. *Int J Dermatol* 1984;23:283.
- 92 Hendren S: Identical alopecia areata in identical twins. *Arch Dermatol* 1949;60:793–795.
- 93 Weidman AI, Zion LS, Mamelok AE: Alopecia areata occurring simultaneously in identical twins. *Arch Dermatol* 1956;74:424–426.
- 94 Werth VP, White WL, Sanchez MR, Franks AG: Incidence of alopecia areata and lupus erythematosus. *Arch Dermatol* 1992;128:368–371.
- 95 Insler MS, Helm CJ: Alopecia areata including the cilia and eyebrows of two sisters. *Ann Ophthalmol* 1989;21:451–453.
- 96 Hordinsky MK, Hallgren H, Nelsen D, Filipovich AH: Familial alopecia areata. HLA antigens and autoimmunity formation in an American family. *Arch Dermatol* 1984;120:464–468.
- 97 Sharma VK, Dawn G, Kumar B: Profile of alopecia areata in Northern India. *Int J Dermatol* 1996;35:22–27.
- 98 Du Vivier A, Munro DD: Alopecia areata, autoimmunity, and Down's syndrome. *Br Med J* 1975;1:191–192.
- 99 Carter DM, Jegasothy BV: Alopecia areata and Down syndrome. *Arch Dermatol* 1976;112:1397–1399.
- 100 Tazi-Ahnini R, Cork MJ, Gawkrödger DJ, Birch MP, Wengraf D, McDonagh AJG, Messenger AG: Role of the autoimmune regulator (AIRE) gene in alopecia areata: strong association of a potentially functional AIRE polymorphism with alopecia universalis. *Tissue Antigens* 2002;60:489–495.

- 101 Theofilopoulos AN: The basis of autoimmunity. Part II. Genetic predisposition. *Immunol Today* 1995;16:150–159.
- 102 Blum A, Miller H: The major histocompatibility complex and inflammation. *Southern Med J* 2000;93:169–171.
- 103 Campbell RD, Milner CM: MHC genes in autoimmunity. *Curr Opin Immunol* 1993;5:887–893.
- 104 Corzo D, Salazar M, Granja CB, Yunis EJ: Advances in HLA genetics. *Exp Clin Immunogenet* 1995;12:156–170.
- 105 Tomlinson IP, Bodmer WF: The HLA system and the analysis of multifactorial genetic disease. *Trends Genet* 1995;11:493–498.
- 106 Thompson G: HLA disease associations: models for the study of complex human genetic disorders. *Crit Rev Clin Lab Sci* 1995;32:183–219.
- 107 Welsh EA, Clark HH, Epstein SZ, Reveille JD, Duvic M: Human leukocyte antigen-DQB1 *03 alleles are significantly associated with alopecia areata. *J Invest Dermatol* 1994;103:758–763.
- 108 Morling N, Frentz G, Fugger L, Georgsen J, Jakobsen B, Odum N, Svejgaard A: DNA polymorphism of HLA class II genes in alopecia areata. *Dis Markers* 1991;9:35–42.
- 109 Kavak A, Baykal C, Ozarmagan G, Akar U: HLA and alopecia areata. *Int J Dermatol* 2000;39:589–592.
- 110 Kianto U, Reunala T, Karvonen J, Lassus A, Tiilikainen A: HLA-B12 in alopecia areata. *Arch Dermatol* 1977;113:1716.
- 111 Kuntz BM, Selzle D, Braun-Falco O, Scholz S, Albert ED: HLA antigens in alopecia areata. *Arch Dermatol* 1997;113:1717.
- 112 Hacham-Zadeh S, Brautbar C, Cohen C, Cohen T: HLA and alopecia areata in Jerusalem. *Tissue Antigens* 1981;18:71–74.
- 113 Averbakh EV, Pospelov LE: HLA antigens in patients with alopecia areata. *Vestn Dermatol Venerol* 1986;1:24–26.
- 114 Frentz G, Thomsen K, Jakobsen BK, Svejgaard A: HLA-DR4 in alopecia areata. *J Am Acad Dermatol* 1986;14:129–130.
- 115 Orecchia G, Belvedere MD, Martinetti M, Capelli E, Rabbiosi G: Human leukocyte antigen region involvement in the genetic predisposition to alopecia areata. *Dermatologica* 1987;175:10–14.
- 116 Duvic M, Hordinsky MK, Fiedler VC, O'Brien WR, Young R, Reveille JD: HLA-D locus associations in alopecia areata: DRw52a may confer disease resistance. *Arch Dermatol* 1991;127:64–68.
- 117 Duvic M, Welsh EA, Jackow C, Papadopoulos E, Reveille JD, Amos C: Analysis of HLA-D locus alleles in alopecia areata patients and families. *J Invest Dermatol* 1995;104(suppl):5S–6S.
- 118 Colombe BW, Price VH, Khoury EL, Lou CD: HLA class II alleles in long-standing alopecia totalis/alopecia universalis and long-standing patchy alopecia areata differentiate these two clinical groups. *J Invest Dermatol* 1995;104(suppl):4S–5S.
- 119 Barahmani N, deAndrade M, Slusser JP, Zhang Q, Duvic M: Major histocompatibility complex class I chain-related gene A polymorphisms and extended haplotypes are associated with familial alopecia areata. *J Invest Dermatol* 2006;126:74–78.
- 120 Hang L, Nakamura RM: Current concepts and advances in clinical laboratory testing for autoimmune diseases. *Crit Rev Clin Lab Sci* 1997;34:275–311.
- 121 Martinez-Mir A, Zlotogorski A, Gordon D, Petukhova L, Mo J, Gilliam TC, Londono D, Haynes C, Ott J, Hordinsky M, Nanova K, Norris D, Price V, Duvic M, Christiano AM: Genomewide scan for linkage reveals evidence of several susceptibility loci for alopecia areata. *Am J Hum Genet* 2007;80:316–328.
- 122 Gollinck H, Orfanos CE: Alopecia areata: pathogenesis and clinical picture; in Orfanos CE, Happle R: *Hair and Hair Diseases*. Berlin, Springer-Verlag, 1990, pp 529–569.
- 123 Price VH, Khoury EL: Alopecia areata. *Prog Dermatol* 1991;25:1–7.
- 124 Conroy JD: Alopecia areata; in Andrews EJ, Ward BC, Altman NH: *Spontaneous Animal Models of Human Disease*. New York, Academic Press, 1979, pp 30–31.
- 125 Muller GH, Kirk RW, Scott DW: *Small Animal Dermatology*, ed 3. Philadelphia, WB Saunders, 1983.

- 126 Paradis M, Fecteau G, Scott DW: Alopecia areata (pelade) in a cow. *Can Vet J* 1988;29:727–729.
- 127 Scott DW, Guard CL: Alopecia areata in a cow. *Agri-Practice* 1998;9:16–19.
- 128 Sundberg JP, Oliver RF, McElwee KJ, King LE: Alopecia areata in humans and other mammalian species. *J Invest Dermatol* 1995;104:32S–33S.
- 129 Smyth JR, McNeil M: Alopecia areata and universalis in the Smyth chicken model for spontaneous autoimmune vitiligo. *J Invest Dermatol Symp Proc* 1999;4:211–215.
- 130 Michie HJ, Jahoda CAB, Oliver RF, Poulton TA: Immunobiological studies on the alopecic (DEBR) rat. *Br J Dermatol* 1990;123:557–567.
- 131 Michie HJ, Jahoda CAB, Oliver RF, Johnson BE: The DEBR rat: an animal model of human alopecia areata. *Br J Dermatol* 1991;125:94–100.
- 132 Oliver R, Jahoda CAB, Horne KA, Michie HJ, Poulton T, Johnson BE: The DEBR rat model for alopecia areata. *J Invest Dermatol* 1991;96:97S.
- 133 Yanagisawa H, Richardson JA, Taurog JD, Hammer RE: Characterization of psoriasiform and alopecic skin lesions in HLA-B27 transgenic rats. *Am J Pathol* 1995;147:995–964.
- 134 Sundberg JP, Vallee CM, King LE: Alopecia areata in aging C3H/HeJ mice; in Sundberg JP: *Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools*. Boca Raton, CRC Press, 1994, pp 499–505.
- 135 McElwee KJ, Boggess D, King LE, Sundberg JP: Experimental induction of alopecia areata-like hair loss in C3H/HeJ mice using full-thickness skin grafts. *J Invest Dermatol* 1998;111:797–803.
- 136 Friedman PS: Clinical and immunological association of alopecia areata. *Semin Dermatol* 1985;4:9–13.
- 137 Tobin DJ, Fenton DA, Kendall MD: Ultrastructural observations on the hair bulb melanocytes and melanosomes in acute alopecia areata. *J Invest Dermatol* 1990;94:803–807.
- 138 Tobin DJ, Fenton DA, Kendall MD: Cell degeneration in alopecia areata: an ultrastructural study. *Am J Dermatopathol* 1991;13:248–256.
- 139 Shapiro J, Sundberg JP, Bissonnette R, McElwee KJ, McLean DI, Carroll JM, Oliver RF, Tang L, Lui H: Alopecia areata-like hair loss in C3H/HeJ mice and DEBR rats can be reversed using topical diphencyprone. *J Invest Dermatol Symp Proc* 1999;4:239.
- 140 Freyschmidt-Paul P, Metz S, Sundberg JP, Happle R, Hoffmann R: Induction of hair regrowth in C3H/HeJ alopecia areata by treatment with the topical immunosuppressant FK506. *J Invest Dermatol* 2000;114:871.
- 141 Freyschmidt-Paul P, Hoffmann R, Levin R, Sundberg JP, Happle R, McElwee KJ: Current and potential therapeutic agents for the treatment of alopecia areata. *Cur Pharm Des* 2001;7: 213–230.
- 142 Gardner SP, Freyschmidt-Paul P, Hoffmann R, Sundberg JP, Happle R, Linsey NJ, Tobin DJ: Normalisation of hair follicle morphology in C3H/HeJ alopecia areata mice after treatment with squaric acid dibutylester. *Eur J Dermatol* 2000;10:443–450.
- 143 Ahmad W, Faiyaz U, Brancolini V, Tsou HC, Haque Su, Lam H, Alta VM, Owen J, deBlaquiere M, Frank J, Cserhalmi-Friedman PB, Leask A, McGrath JA, Peacocke M, Ahmad M, Ott J, Christiano AM: Alopecia universalis associated with a mutation in the human hairless gene. *Science* 1998;279:720–724.
- 144 Sundberg JP, Dunstan RW, Compton JG: *Hairless Mouse, HRS/J hr/hr*. Heidelberg, Springer-Verlag, 1989.
- 145 Sundberg JP: The hairless (*hr*) and rhino (*hr^{rh}*) mutations, chromosome 14; in Sundberg JP: *Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools*. Boca Raton, CRC Press, 1994, pp 291–312.
- 146 Sundberg JP, Price VH, King LE: The 'hairless' gene in mouse and man. *Arch Dermatol* 1999;135:718–720.
- 147 Ahmad W, Panteleyev A, Sundberg JP, Christiano AM: Molecular basis for the rhino-8J mouse mutation: a nonsense mutation in the mouse hairless gene. *Genomics* 1998;53:383–386.
- 148 Christiano AM, Irvine A, McGrath JA, Ahmad M, Henson-Apollonio V, Sundberg JP, hmad W: Molecular pathology of papular atrichia: hairless gene mutations in humans and rhino mice. *Third International Alopecia Areata Workshop*, Washington, 1998.
- 149 Panteleyev AA, Ahmad W, Malashenko AM, Ignatieva EL, Paus R, Sundberg JP, Christiano AM: Molecular basis for the rhino Yurlovo (*hr^{rh-Y}*) phenotype: severe skin abnormalities and female

- reproductive defects associated with an insertion in the hairless gene. *Exp Dermatol* 1998;7: 281–288.
- 150 Ahmad WA, Panteleyev AA, Henson-Apollonio V, Sundberg JP, Christiano AM: Molecular basis of a novel rhino (*hr^{rh-Chr}*) phenotype: a nonsense mutation in the mouse hairless gene. *Exp Dermatol* 1998;7:298–301.
 - 151 Panteleyev AA, Paus R, Ahmad W, Sundberg JP, Christiano AM: Molecular and functional aspects of the hairless (*hr*) gene in laboratory rodents and humans. *Exp Dermatol* 1998;7:249–267.
 - 152 Ahmad W, Panteleyev AA, Christiano AM: The molecular basis of congenital atrichia in humans and mice: mutations in the hairless gene. *J Invest Dermatol Symp Proc* 1999;4:240–243.
 - 153 McElwee KJ, Boggess D, King LE, Sundberg JP: Alopecia areata versus juvenile alopecia in C3H/HeJ mice: tools to dissect the role of inflammation in focal alopecia. *Exp Dermatol* 1999;8:354–355.
 - 154 Bullard DC, Scharffetter-Kochanek K, McArthur MJ, Chosay JG, McBride ME, Montgomery CA, Beaudet AL: A polygenic mouse model of psoriasiform skin disease in CD18-deficient mice. *Proc Natl Acad Sci USA* 1996;93:2116–2121.
 - 155 McElwee KJ, Silva K, Beamer WG, King LE, Sundberg JP: Melanocyte and gonad activity as potential modifying factors in C3H/HeJ mouse alopecia areata. *Exp Dermatol* 2001;10: 420–429.
 - 156 Oh HS, Smart RC: An estrogen receptor pathway regulates the telogen-anagen hair follicle transition and influences epidermal differentiation. *Proc Natl Acad Sci USA* 1996;93: 12525–12530.
 - 157 Stenn KS, Paus R, Filippi M: Failure of topical estrogen receptor agonists and antagonists to alter murine hair follicle cycling. *J Invest Dermatol* 1998;110:95.
 - 158 Smart RC, Oh H-S, Robinette CL: Effects of 17-B-Estradiol and ICI 182 780 on hair growth in various strains of mice. *J Invest Dermatol Symp Proc* 1999;4:285–289.
 - 159 Ohnemus U, Uenalani M, Inzunza J, Gustafsson JA, Paus R: The hair follicle as an estrogen target and source. *Endocr Rev* 2006;27:677–706.
 - 160 Gardner SH: The role of autoantibodies to hair follicles in alopecia areata. Thesis, University of Bradford, 2001.
 - 161 Sundberg JP, Orlow SJ, Sweet HO, Beamer WG: The adrenocortical dysplasia (*acd*) mutation, Chromosome 8; in Sundberg JP: *Handbook of Mouse Mutations with Skin and Hair Abnormalities: Animal Models and Biomedical Tools*. Boca Raton, CRC Press, 1994, pp 159–164.
 - 162 Epidemiological evidence of the association between lichen planus and two immune-related diseases: alopecia areata and ulcerative colitis. Gruppo Italiano Studi Epidemiologici in Dermatologia. *Arch Dermatol* 1991;127:688–691.
 - 163 Treem WR, Veligati LN, Rotter JI, Targan SR, Hyams JS: Ulcerative colitis and total alopecia in a mother and her son. *Gastroenterology* 1993;104:1187–1191.
 - 164 Brandwein SL, McCabe RP, Cong Y, Waites KB, Ridwan BU, Dean PA, Ohkusa T, Birkenmeier EH, Sundberg JP, Elson EO: Spontaneously colitic C3H/HeJBir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora. *J Immunol* 1997;159:44–52.
 - 165 Gilhar A, Ullmann Y, Berkutzi T, Assy B, Kalish R: Autoimmune hair loss (alopecia areata) transferred by T lymphocytes to human scalp explants on SCID mice. *J Clin Invest* 1998;101: 62–67.
 - 166 Zoller M, McElwee KJ, Engel P, Hoffmann R: Transient CD44 variant isoform expression and reduction in CD4+/CD25+ regulatory T cells in C3H/HeJ mice with alopecia areata. *J Invest Dermatol* 2002;118:983–992.
 - 167 McElwee KJ, Silva F, Boggess D, Bechtold L, King, LE Jr, Sundberg JP: Alopecia areata in C3H/HeJ mice involves leukocyte-mediated root sheath disruption in advance of overt hair loss. *Vet Pathol* 2003;40:643–650.
 - 168 Hara M, Kingsley CI, Niimi M, Read S, Turvey SE, Bushell AR, Morris PJ, Powrie F, Wood KJ: IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. *J Immunol* 2001;166:3789–3796.
 - 169 Kumanogoh A, Wang X, Lee I, Watanabe C, Kamanaka M, Shi W, Yoshida K, Sato T, Habu S, Itoh M, Sakaguchi N, Sakaguchi S, Kikutani H: Increased T cell autoreactivity in the absence of CD40-CD40 ligand interactions: a role of CD40 in regulatory T cell development. *J Immunol* 2001; 166:353–360.

- 170 Tang L, Lui H, Sundberg JP, Bissonnette R, McLean DI, Shapiro J: Restoration of hair growth with topical diphencyprone in mouse and rat models of alopecia areata. *J Am Acad Dermatol* 2003;49:1013–1019.
- 171 Freyschmidt-Paul P, Seiter S, Zoller M, Konig A, Zeigler A, Sundberg JP, Happle R, Hoffmann R: Treatment with an anti-CD44v10-specific antibody inhibits the onset of alopecia areata in C3H/HeJ mice. *J Invest Dermatol* 2000;115:653–657.
- 172 Grabbe S, Varga G, Beissert S, Steinert M, Pendl G, Seeliger S, Bloch W, Peters T, Schwarz T, Sunderkotter C, Scharffetter-Kochanek K: B2 integrins are required for skin homing of primed T cells but not for priming naive T cells. *J Clin Invest* 2002;109:183–192.
- 173 Borson ND, Strausbauch MA, Kennedy RB, Oda RP, Landers JP, Wettstein PJ: Temporal sequence of transcription of perforin, fas ligand, and tumor necrosis factor- α genes in rejecting skin allografts. *Transplantation* 1999;67:672–680.
- 174 Dieleman LA, Elson CO, Tennyson GS, Beagley KW: Kinetics of cytokine expression during healing of acute colitis in mice. *Am J Physiol* 1996;271:G130–G136.
- 175 Hubner G, Brauchle M, Smola H, Madlener M, Fassler R, Werner S: Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. *Cytokine* 1996;8:548–556.
- 176 Parenteau GL, Doherty GM, Peplinski GR, Tsung K, Norton JA: Prolongation of skin allografts by recombinant tumor necrosis factor and interleukin-1. *Ann Surg* 1995;221:572–578.
- 177 Cumberbatch M, Kimber I: Dermal tumour necrosis factor- α induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhans' cell migration. *Immunology* 1992;75:257–263.
- 178 Cumberbatch M, Fielding I, Kimber I: Modulation of epidermal Langerhans' cell frequency by tumor necrosis factor- α . *Immunology* 1994;81:395–401.
- 179 Cotran RS, Pober JS: Cytokine-endothelial interactions in inflammatory immunity and vascular injury. *J Am Soc Nephrol* 1990;1:225–235.
- 180 Poper JS, Cotran RS: Cytokines and endothelial cell biology. *Physiol Rev* 1990;70:427–451.
- 181 Kainulainen V, Nelimarkka L, Jarvelainen H, Laato M, Jalkanen M, Elenius K: Suppression of syndecan-1 expression in endothelial cells by tumor necrosis factor- α . *J Biol Chem* 1996;271:18759–18766.
- 182 Slowik MR, DeLuca LG, Fiers W, Pober JS: Tumor necrosis factor activates human endothelial cells through the p55 tumor necrosis factor but the p75 receptor contributes to activation at low tumor necrosis factor concentration. *Am J Pathol* 1993;143:1724–1730.
- 183 Galbraith GMP, Pandey JP: Tumor necrosis factor α (TNF- α) gene polymorphism in alopecia areata. *Hum Genet* 1995;96:433–436.
- 184 Bugeon L, Dallman MJ: Costimulation of T cells. *Am J Respir Crit Care Med* 2000;162:S164–S168.
- 185 Sievenhaar F, Sharov AA, Peters EM, Sharova TY, Syska W, Mardaryev AN, Freyschmidt-Paul P, Sundberg JP, Maurer M, Botchkarev VA: Substance P as an immunomodulatory neuropeptide in a mouse model for autoimmune hair loss (alopecia areata). *J Invest Dermatol* 2007;127:1389–1497.
- 186 McElwee KJ, Freyschmidt-Paul P, Hoffmann R, Kissling S, Hummel S, Vitacolonna M, and Zoller M: Transfer of CD8+ cells induces localized hair loss whereas CD4+/CD25– cells promote systemic alopecia areata and CD4+/CD25+ cells blockade disease onset in the C3H/HeJ mouse model. *J Invest Dermatol* 2005;124:947–957.
- 187 Tobin DJ, Olivry T, Sundberg JP, Boggess D, King LE, Bystryń J-C: Hair follicle-specific antibodies in mammalian species with alopecia areata. *J Invest Dermatol* 1997;108:654.
- 188 Kuwano Y, Fujimoto M, Watanabe R, Asashima N, Nakashima H, Ohno H, Yano S, Yazawa N, Okochi H, Tamaki K: Serum anti-Fc γ receptor autoantibodies in patients with alopecia areata. *Arch Dermatol Res* 2007;298:493–498.
- 189 Sundberg JP, Boggess D, Silva KA, McElwee KJ, King LE, Li R, Churchill G, Cox GA: Major locus on mouse chromosome 17 and minor locus on chromosome 9 are linked with alopecia areata in C3H/HeJ mice. *J Invest Dermatol* 2003;120:771–775.
- 190 Sundberg JP, Silva KA, Li R, King LE, Cox GA: Adult onset alopecia areata is a complex polygenic trait in the C3H/HeJ mouse model. *J Invest Dermatol* 2004;123:294–297.

- 191 Freyschmidt-Paul P, Sundberg JP, Happle R, McElwee KJ, Metz S, Boggess D, Hoffmann R: Successful treatment of alopecia areata-like hair loss with the contact sensitizer squaric acid dibutylester (SADBE) in C3H/HeJ mice. *J Invest Dermatol* 1999;113:61–68.
- 192 Schafer N, Blaumeiser B, Becker T, Freudenberg-Hua Y, Eigelshoven HS, Schmael C, Lambert J, DeWeert J, Kruse R, Nothen MM, Betz RC: Investigation of the functional variant c.-169T>C of the Fc receptor-like 3 (FCRL3) gene in alopecia areata. *Int J Immunogenet* 2006;33: 393–395.
- 193 Colombe BW, Lou CD, Price VH: The genetic basis of alopecia areata: HLA associations with patchy alopecia areata versus alopecia totalis and alopecia universalis. *J Invest Dermatol Symp Proc* 1999;4:216–219.
- 194 deAndrade M, Jackow CM, Dahm N, Hordinsky M, Reveille JD, Duvic M: Alopecia areata in families: association with the HLA locus. *J Invest Dermatol Symp Proc* 1999;4:220–223.
- 195 Xiao FL, Zhou FS, Liu JB, Yan KL, Cui Y, Gao M, Liang YH, Sun LD, Zhou SM, Zhu YG, Zhang XJ, Yang S: Association of HLA-DQA1 and DQB1 alleles with alopecia areata in Chinese Hans. *Arch Dermatol Res* 2005;297:201–209.
- 196 Xiao FL, Yang S, Yan KL, Cui Y, Liang YH, Zhou FS, Du WH, Gao M, Sun LD, Fan X, Chen JJ, Wang PG, Zhu YG, Zhou SM, Zhang XJ: Association of HLA class I alleles with alopecia areata in Chinese Hans. *J Dermatol Sci* 2006;41:109–119.
- 197 Marques Da Costa C, Dupont E, VanderCruys M, Andrien M, Hidajat M, Song M, Stene JJ: Earlier occurrence of severe alopecia areata in HLA-DRB1*11-positive patients. *Dermatology* 2006;213:12–14.
- 198 Tarlow JK, Clay FE, Cork MJ, Blakemore AI, McDonagh AJ, Messenger AG, Duff GW: Severity of alopecia areata is associated with a polymorphism in the interleukin-1 receptor antagonist gene. *J Invest Dermatol* 1994;103:387–390.
- 199 Galbraith GM, Palesch Y, Gore EA, Pandey JP: Contribution of interleukin 1 β and KM loci to alopecia areata. *Hum Hered* 1999;49:85–89.
- 200 Tazi-Ahnini R, McDonagh AJ, Cox A, Messenger AG, Britton JE, Ward SJ, Bavik CO, Duff GW, Cork MJ: Association analysis of IL1A and IL1B variants in alopecia areata. *Heredity* 2001;87:215–219.
- 201 Cox A, Camp NJ, Nicklin MJH, diGiovine FS, Duff GW: An analysis of linkage disequilibrium in the interleukin-1 gene cluster, using a novel grouping method for multiallelic markers. *Am J Hum Genet* 1998;62:1180–1188.
- 202 Galbraith GMP, Pandey JP: Km1 allotype associated with one subgroup of alopecia areata. *Am J Hum Genet* 1989;44:426–428.
- 203 Dugoujon JM, Guitard E, Senegas MT: Gm and Km allotypes in autoimmune diseases. *G Ital Cardiol* 1992;22:85–95.
- 204 Dugoujon JM, Cambon-Thomsen A: Immunoglobulin allotypes (GM and KM) and their interactions with HLA antigens in autoimmune diseases: a review. *Autoimmunity* 1995;22:245–260.
- 205 Tazi-Ahnini R, McDonagh A, diGiovine F, Messenger A, Amadou C, Cox A, Duff G, Cork M: Structure and polymorphism of the human gene for the interferon-induced p78 protein (MX1): alopecia areata association with the Down syndrome region. *J Invest Dermatol* 2000; 114:827.
- 206 Gilhar A, Shalaginov R, Assy B, Serafimovich S, Kalish RS: Alopecia areata is a T-lymphocyte mediated autoimmune disease: lesional human T-lymphocytes transfer alopecia areata to human skin grafts on SCID mice. *J Invest Dermatol Symp Proc* 1999;4:207–210.
- 207 Tobin DJ, Gardner SH, Luther PB, Dunston SM, Lindsey NJ, Olivry T: A natural canine homologue of alopecia areata in humans. *Br J Dermatol* 2003;149:938–950.
- 208 Noli C, Toma S: Three cases of immune-mediated adnexal skin disease treated with cyclosporine. *Vet Dermatol* 2006;17:85–92.
- 209 Conroy JD: An overview of immune-mediated mucocutaneous diseases in the dog and cat. II. Other diseases based on immunologic mechanisms. *Am J Dermatopathol* 1983;5:595–599.
- 210 Colombo S, Keen JA, Brownstein DG, Rhind SM, McGorum BC, Hill PB: Alopecia areata with lymphocytic mural folliculitis affecting the isthmus in a thoroughbred mare. *Vet Dermatol* 2004;15:260–265.

- 211 Shresta S, Smyth JR, Erf GF: Profiles of pulp infiltrating lymphocytes at various times throughout feather regeneration in Smyth line chickens with vitiligo. *Autoimmunity* 1997;25:193–201.
- 212 Wick G, Andersson L, Hala K, Gershwin ME, Selmi C, Erf GF, Lamont SJ, R RS: Avian models with spontaneous autoimmune diseases. *Adv Immunol* 2006;92:71–117.
- 213 McElwee KJ, Boggess D, Olivry T, Oliver RJ, Whiting D, Tobin JD, Bystry J-C, King LE, Sundberg JP: Comparison of alopecia areata in human and nonhuman mammalian species. *Pathobiology* 1998;66:90–107.

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Dermatomyositis

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Abstract

Dermatomyositis (DM) is a chronic inflammatory disorder of the skin and muscles. Although thought to be autoimmune in origin, many questions remain as to the etiopathogenesis of this disease. DM has classically been considered a humorally mediated disease. Current evidence, however, seems to increasingly support alternative (though not mutually exclusive) mechanisms of pathogenesis, including cell-mediated and innate immune system dysfunction. Pathologic findings of DM in muscle include infarcts, perifascicular atrophy, endothelial cell swelling and necrosis, vessel wall membrane attack complex deposition, and myocyte-specific MHC I upregulation. As for the skin, histopathologic findings include hyperkeratosis, epidermal basal cell vacuolar degeneration and apoptosis, increased dermal mucin deposition, and a cell-poor interface dermatitis. Autoantibodies, particularly those that bind nuclear or cytoplasmic ribonucleoprotein antigens, are also commonly found in DM, although their importance in pathogenesis remains unclear. Defective cellular clearance, genetic predilection and environmental exposures, such as viral infection, may also play an important role in the pathogenesis of DM. The seminal work regarding the pathogenesis of DM is reviewed and an update on the recent basic and molecular advances in the field is provided.

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Dermatomyositis (DM) is a chronic inflammatory disorder of the skin and muscles. Although thought to be autoimmune in origin, many questions remain as to the etiopathogenesis of this disease. Adult-onset DM can start at any age and generally affects females three times as frequently as males [1]. Symptoms usually manifest themselves in both the skin and muscle during the initial acute attack. Muscle symptoms, which include weakness, myalgias or tenderness, more frequently involve the proximal muscles [1]. The skin usually presents with at least one of a number of characteristic features, including Gottron's papules over the metacarpophalangeal, proximal and distal interphalangeal,

elbow and knee joints, a heliotrope rash around the eyes, periungual telangiectasias, and dystrophic cuticles [2]. DM is associated with an increased risk of internal malignancy, including neoplasms of the gastrointestinal tract, ovary and breast [3, 4]. An amyopathic subset of DM in which subjects do not experience muscle weakness or myalgias is well described [5]. A recent review of amyopathic DM shows that this group may also be at increased risk of malignancy and interstitial lung disease (ILD) as those with clinical features of muscle disease [6]. In this chapter we present a review of the seminal work regarding the pathogenesis of DM and provide an update on the recent basic and molecular advances in the field.

Pathogenesis

Muscle

DM is currently viewed as a humorally mediated autoimmune disease in which antigen-specific antibodies are deposited in the microvasculature, either secondary to immune complex deposition or specific anti-endothelial cell binding [2, 7]. C1 or C3 activation then follows, leading to C5b-9 membrane attack complex (MAC) deposition in the walls of the blood vessels. The present model implicates MAC deposition in capillary necrosis, perivascular inflammation and infiltration of muscle by B cells, which theoretically results in endofascicular hypoperfusion, muscle ischemia and perifascicular atrophy [7, 8]. Complement activation also results in cytokine and chemokine release, which recruits CD4+ T cells and macrophages to the affected muscle tissue. While this is an attractive model, it remains unproven.

Muscle disease is a common and bothersome element of DM. Although clinically amyopathic forms of DM are well described [5, 6], the extent of muscle dysfunction on the microscopic or molecular level in this subgroup is not well described. Evidence suggests that muscle pathology may still be present despite the absence of key findings on biopsy, including inflammation, tubuloreticular inclusions and MAC deposition highlighted by immunohistochemistry [9]. In evaluating the genetic expression profile of the idiopathic inflammatory myopathies, Greenberg et al. [9] found that the myocyte genetic expression profile in at least one DM patient with a normal muscle biopsy was shown to be similar to that seen in those with classic DM.

The typical histopathology findings of DM in muscle include infarcts, perifascicular atrophy, endothelial cell swelling, vessel wall MAC deposition, capillary necrosis, major histocompatibility complex (MHC) I upregulation, and the presence of an inflammatory infiltrate consisting of T and B lymphocytes, macrophages and plasma cells [10]. DM has classically been considered a

humorally mediated disease in part because of these findings, specifically the predominant perimysial and perivascular B/CD4+ T cell infiltrate and intravascular MAC deposition. Current evidence, however, seems to increasingly support alternative (though not mutually exclusive) mechanisms of pathogenesis, including cell-mediated and innate immune system dysfunction.

Plasmacytoid dendritic cells (pDCs) provide immune surveillance and serve as a link between the adaptive and innate arms of the immune system [11]. Recent studies provide evidence that pDCs are dysregulated in muscle samples of DM patients [8, 12]. More than 30–90% of the CD4+ cells found in DM muscle are pDCs [8]. Page et al. [12] report the presence of immature, CD1a+ pDCs localized in perivascular infiltrates within the endomysium. In this study, immature pDCs were thought to be recruited by CCL20 upregulation. Mature pDCs were also found in high numbers, though they are thought to result from *in situ* maturation of immature pDCs instead of mature pDC extravasation. Dysregulated pDCs are thought to release type I interferons (IFNs) locally, likely resulting in widespread effects. In examining the gene expression profile of DM muscle, most of the highly upregulated genes include IFN- α/β -inducible gene promotor sequences [8]. The protein products of these IFN- α/β -responsive genes have been shown to mediate myofiber and endothelial cell injury [8]. Elevated levels of the IFN- α -responsive gene MxA have been found in DM muscle samples and may play a role in muscle damage and inflammation [8]. For example, Greenberg and Amato [7] and Greenberg et al. [8] have suggested that MxA may be the primary component of the tubuloreticular inclusion, a characteristic pathologic feature of DM which may be involved in endothelial dysfunction. If true, MxA overexpression could provide the link between the damaging effects of tubuloreticular inclusions and the dysregulated arm of innate immunity [8]. All of these findings suggest that DM may be caused by a dysregulation of innate immunity to an unknown trigger.

Contrary to current dogma, T cell-mediated cytotoxicity may play a role in the muscle inflammation and damage in DM. Bank et al. [13] have shown that *in vitro* cell-mediated myocytotoxic damage induced by autologous peripheral blood CD3+ cell clones isolated from DM patients can be blocked with OKT3, an anti-CD3 molecule. Furthermore, perivascular perforin-positive CD8 cells have been identified in muscle samples taken from a group of DM subjects, which also supports the role for T cell-mediated cytotoxicity [14]. As CD4+ pDCs may also stimulate the adaptive arm of the immune system, it is likely that multiple arms of the immune system contribute to the pathogenesis of DM [11].

Other cytokines of importance have also been demonstrated. IL-4 is increased at perimysial sites, potentially secondary to the increased number of perimysial CD4+ T lymphocytes [15]. Upregulation of IL-17 and IFN- γ has been reported in DM muscle as well [8].

Certain cell-trafficking molecules have also been examined in DM. Increased expression of monocyte chemoattractant protein 1 (MCP-1), a regulator of lymphocyte trafficking, has been shown in endomysial and perifascicular arterioles and capillaries [16]. Upregulated by TNF- α , MCP-1 was also found expressed in some control samples, suggesting its possible role in normal immune surveillance. A recent linkage analysis, however, did not identify a strong genetic association of MCP-1 gene polymorphisms and DM [17]. The cell-trafficking regulators CXCR2 and IP-10 (a CXCR3 ligand) have been found in perimysial blood vessels and infiltrates of DM, suggesting a Th1-mediated disease process [18]. Furthermore, increased levels of the cell adhesion molecules VCAM-1 and ICAM-1 have been shown within the muscle fibers, perimysial arteries and perimysial venules of DM muscle biopsies [15, 19].

Role of MHC I in Muscle

The expression of MHC I, which is generally not expressed in adult myocytes, is upregulated in DM [20, 21], specifically in type II muscle fibers [22]. Karpati et al. [20] specifically noted upregulation of MHC I in perifascicular myocytes in conjunction with other associated morphological changes within the muscle fibers (atrophy, z-disc streaming, punched out myofibrillar areas and mitochondrial maldistribution). Described as an early event preceding the inflammatory infiltrate, MHC I upregulation may result from mononuclear cell-cytokine-independent mechanisms [23]. Others have postulated that muscle damage secondary to capillary dropout, ischemia and the subsequent reparative process, viral infection, IFNs, prostaglandins or cellular stress may lead to MHC I upregulation [20]. Pavlath [21] has shown in a muscle freeze injury mouse model that IFN- γ can induce MHC I expression in regenerating myocytes. This explains why MHC I may be upregulated after the cycle of damage and regeneration has been initiated, but does not account for the early upregulation. Nevertheless, Pavlath [21] suggests that the MHC I upregulation in regenerating myofibers in the setting of perifascicular inflammation implicates T cells in the pathogenesis of DM. It does seem, however, that understanding the timing of MHC I upregulation will be important in ultimately describing the pathogenesis of DM. For example, the overexpression of MHC I in skeletal muscles of adult mice initiates what seems to be a self-sustaining autoimmune myositis more similar to polymyositis (PM) than DM, where the perivascular inflammation or perifascicular atrophy seen in DM was not found to be present [23]. MHC I upregulation may directly contribute to the muscle damage in DM via activation of a cytotoxic T cell host response.

Although other immune-mediated mechanisms of muscle damage (MHC I cross-presentation by dendritic cells to CD4 or direct autoantibody binding) are also plausible, Nagaraju et al. [24] have proposed a unique mechanism for the

myocyte damage seen in DM. They postulated that MHC I upregulation itself, independent of cytotoxic mediator cells, may directly induce cell death via the endocytoplasmic reticulum (ER) stress response. According to this proposal, the ER overload response and unfolded protein response, which together comprise the ER stress response, are initiated after MHC class I overexpression – a response to some unknown stimulation (such as transgene induction, infection or denervation). The downstream events of the ER stress response were shown in this study to upregulate nuclear factor κ B and its targets (ICAM-1, VCAM, β_2 -microglobulin), the ER chaperone protein GRP 78, and caspase-12, a critical mediator of cell death [24].

Skin

Although much effort has focused on muscle pathology in DM, studies have recently shed light on the pathogenesis of skin disease. The study of skin pathogenesis will be important, as specific cutaneous findings may be the common denominator for this disease. The classic findings of Gottron's papules, heliotrope rash, mechanics hands, macular violaceous erythema (shawl sign, v-neck rash, holster sign) and nailfold telangiectasia are frequently seen with DM, even in those lacking muscle symptoms [5]. The importance of skin disease in the ultimate pathogenesis of DM seems especially important, given that the risk profile for malignancy and ILD seen between the amyopathic and classic subtypes may be similar [6].

The typical cutaneous histopathologic changes in DM include hyperkeratosis, epidermal basal cell vacuolar degeneration, pathologic apoptosis of epidermal basal and suprabasal cells, dyskeratosis, a focally thinned epidermis, and increased dermal mucin deposition [25–27]. A cell-poor interface dermatitis comprised of lymphocytes at the dermal-epidermal junction is also characteristic [28]. Vascular ectasia and fibrin deposition, C5b-9 deposition in both dermal vasculature and the dermal-epidermal junction, and a perivascular lymphocytic infiltrate are features which are also commonly seen in DM but not cutaneous lupus erythematosus (CLE) [28]. The superficial vascular plexus density has been reported to be lower in myopathic than amyopathic DM, suggesting that these two subsets are at different stages of a common pathogenic process or experience a separate but related sequence of pathologic events [28]. As in muscle, dysregulated cytokine production and cell-mediated immune mechanisms, among other pathologic findings, are thought to contribute to the cutaneous pathogenesis of DM.

Recent evidence from studies of the skin suggests a prominent role of the T cell-mediated immune response. Several studies examining the histopathology of DM lesions have shown that the principal infiltrating cell of DM in the skin is the CD4+ T lymphocyte, distributed mainly in the perivascular upper

dermis [29–31]. The majority of these cells appear to be of activated/memory phenotype [12].

A T cell-mediated host response is also implicated in the pathogenesis of DM in a recent study which suggests active involvement of the CD40/CD40 ligand (CD40L) system within the skin [29]. A significantly increased number of both CD40+ cells (including keratinocytes and mononuclear cells in the dermis), as well as infiltrating CD4+CD40L+ T lymphocytes, is found in skin biopsies from patients with DM [29]. Activation of the CD40/CD40L system may be responsible for upregulation of several proinflammatory molecules, including IL-6, IL-15, IL-8 and MCP-1 [29, 32].

Keratinocyte apoptosis appears to be dysregulated in the skin of DM, though its full role in disease pathogenesis is not yet clear. The exact mechanism for keratinocyte apoptosis is unclear and includes UVB light, Fas-FasL, TNF- α and CD8+ T cell-mediated activation of the apoptosis pathway [33]. Pablos et al. [27] have reported abnormally increased amounts of apoptosis in active skin lesions of CLE and DM, most notably at the basal and suprabasal layers. Consistent with this pathologic finding, the expression of the cell cycle regulator p53 is enhanced in both of these layers [27]. Furthermore, the anti-apoptotic factor Bcl-2 has been shown to be downregulated in the epidermal and follicular basal cell layers [25]. Regardless of mechanism, apoptosis and inflammation at the basal cell layers may increase the likelihood of antigen-presenting cell interaction and T cell stimulation [27].

Photosensitivity is an important clinical feature of DM. Exposure to sunlight and specifically UVA and UVB radiation may serve a central role in disease onset and persistence. Increased levels of apoptosis after exposure to UV radiation have been described in DM and CLE [34, 35]. Kuhn et al. [35] evaluated apoptosis in skin biopsies of 85 CLE patients and noted that defective clearance of apoptotic cells may lead to secondary necrosis, inflammation and enhanced self-antigen presentation. Neoantigen presentation, which has been described in a lupus model following exposure to UV light [36–39], may be involved in DM as well. Although lupus and DM are distinct clinical entities, they share many common pathogenic findings [7, 28, 40, 41]. Exposure to UVB radiation has also been shown to upregulate TNF- α [42], an important proinflammatory cytokine which is increasingly supported by the literature to be involved in DM. An association between TNF- α promoter polymorphisms has been reported in DM [43] and the related diseases subacute CLE [44] and juvenile DM (JDM) [45]. Important differentiating features of DM and CLE, however, have been noted previously. For example, Werth et al. [43] showed no increase in the HLA-DR3 linkage in DM patients with the –308A TNF promoter polymorphism, though one was found in subacute CLE. The association between UVB exposure and TNF- α release is consistent with a model in

which UV light triggers cytokine-mediated inflammation and keratinocyte apoptosis [42].

Type I IFNs (α and β) are thought to play an important role in the pathogenesis of DM as well [30]. One case report of DM following treatment with IFN- α 2b as an adjuvant in the treatment of metastatic melanoma suggests a primary role of type I IFNs in DM [46]. Similar to the pDC dysregulation described in muscle above, Wenzel et al. [30] have reported increased numbers of infiltrating pDCs in DM skin lesions, which are thought to enhance lymphocyte recruitment via type I IFN-mediated upregulation of the CXCR3 ligand IP-10/CXCL10 and subsequent interaction with the lymphocyte CXCR3 receptor. Besides recruiting and activating resting T cells, natural killer cells and monocytes, CXCR3 receptor stimulation is also thought to promote Th1-mediated immunity and inhibit angiogenesis [47]. Although IFN- α itself has not been detected in the skin, IFN- α -dependent proteins (such as MxA) are elevated, which suggests the presence of active IFN- α [30]. Besides its effects on lymphocytes, IFN- α may also be instrumental in causing endothelial cell damage, as is postulated for muscle disease.

Secondary mucinosis, although not as frequently described in DM as in lupus erythematosus, has been reported in a number of subjects with DM [48–50]. Excess mucin deposition in DM has been postulated to occur secondary to increased hyaluronic acid production by dermal fibroblasts following immunological stimulation as opposed to decreased hyaluronidase-mediated resorption [26]. Although IL-1, IL-6, TNF- α , TNF- β and platelet-derived growth factor have all been implicated in increased mucin deposition [51–53], DM-specific studies indicating this are lacking.

Other Mechanisms of Pathogenesis

Autoantibodies

Although autoantigens and autoantibodies are classically thought to be responsible for downstream events of DM, little evidence exists for direct pathogenesis. First, in a study of patients with DM, 44% had sera containing IgM and/or IgG antibodies against endothelial cells, while none of the patients with other myopathies had a positive titer [54]. However, none of the serum from patients with endothelial antibodies had a cytotoxic effect on endothelial cells and cross-reactivity to other tissues was not measured [54]. Second, in a study measuring IgG, IgM and C3 deposits in blood vessels from skeletal muscle of three patients with DM, 100% had immunoglobulin deposits and 33% had C3 deposits in the walls of perimysial veins [55]. However, similar deposits were seen in other inflammatory disorders such as rheumatoid arthritis and

systemic lupus erythematosus (SLE). Third, it is unclear if complement is activated via the classic pathway as proposed or via an alternative pathway, or if immune complexes including MAC are formed within the vasculature and subsequently deposited in the endothelium [7]. Fourth, in a recent review of amyopathic DM, Gerami et al. [6] have shown that myocyte-specific antibodies are less common in amyopathic DM, suggesting that the muscle inflammation and symptoms are a secondary event or a difference in the immunogenic background exists between the groups.

Antibodies to nuclear or cytoplasmic ribonucleoprotein antigens (antisynthetase, anti-signal-recognition particle, anti-Mi-2, anti-PM-Scl and anti-KL6 autoantibodies) are found in approximately 20% of patients with DM [2, 56, 57], but their importance in the etiology and pathogenesis of DM remains unclear. Myositis-specific antibodies, found in patients with DM, PM or inclusion-body myositis, include antibodies to components of the translation machinery, such as aminoacyl tRNA synthetases and RNAs [58]. The most common autoantibody found in myositis patients, anti-Jo-1, targets histidyl-tRNA synthetase (HisRS) and is found in several subsets of patients. Antibodies directed against alanyl-, asparaginyl-, glycyl-, isoleucyl- and threonyl-tRNA synthetases have also been reported in a smaller percentage of patients with DM [59]. Anti-p155 is a newly described autoantibody for DM. Although its target antigen has not yet been identified, anti-p155 was found to be present in 29% of DM patients, whereas it was only seen in 4.2% of PM patients, 2% of SLE patients and in no patients with systemic sclerosis, other myopathies or healthy controls, suggesting it may also be a myositis-specific autoantibody [60].

Anti-Jo-1 antibodies are found associated with ILD, inflammatory arthritis and Raynaud's phenomenon in patients with the anti-tRNA synthetase syndrome [58, 61–64]. For example, in a study of 15 patients with DM or PM and anti-Jo-1 antibodies, 73% presented with ILD, 82% with joint involvement and 55% with Raynaud's phenomenon [65]. However, 60% of patients with ILD and DM had no anti-Jo-1 antibodies, and all patients with ILD had similar therapeutic outcomes. In a study of patients with anti-Jo-1 antibodies, 91% had HLA-DR3 and 80% had HLA-DQ2 loci, indicating a possible common genetic susceptibility [58]. A second group of patients with anti-Jo-1 antibodies have muscle biopsy findings similar to patients with DM, but no capillary loss on muscle biopsy and no cutaneous findings, suggesting that the anti-Jo-1 antibody plays a role in generating some of the phenotypic signs of DM [66]. Immunization of mice with anti-Jo-1 (anti-HisRS), however, was shown to not initiate a generalized myositis [67]. Greenberg et al. [9] have shown that the gene expression profile of muscle in anti-Jo-1 patients is essentially the same as that seen in classic DM. These observations support the theory that the immune response to known autoantigens in DM may be unrelated to the

primary pathogenic mechanisms causing muscle and/or lung disease and may represent epiphenomena [64].

Autoantibody production in DM is currently thought to result from cross-reaction with an infectious agent through molecular mimicry or a breakdown in tolerance to self-antigens [68]. However, neither theory accounts for the limited number of autoantigens described to date in inflammatory myopathies. In order to account for this paucity, Plotz [68] argues that, in myositis, potential autoantigens must possess certain intrinsic structural, biological and immunological properties. The presence of a coiled-coil domain, differential protease cleavage susceptibility and target tissue upregulation of autoantigens, among a variety of other reasons, may determine the autoantibody repertoire. For example, the NH₂-terminal domain of HisRS (anti-Jo-1), a component of translation against which antibodies are formed in approximately 25% of patients with either PM or DM, was shown to be chemotactic for T cells, monocytes and immature dendritic cells *in vitro* [59, 69]. Granzyme B, a serine protease released by activated lymphocytes during the induction of apoptosis, was found to cleave most autoantigens, including HisRS at the NH₂-terminal domain, producing unique fragments [59, 70]. Interestingly, nonautoantigens were either resistant to granzyme B cleavage or did not produce novel fragments which were susceptible to cleavage by other proteases [70]. The authors hypothesize that HisRS, among a host of other myositis-specific autoantigens including asparaginyl-tRNA synthetase, is released from dying cells in sites of tissue damage and inflammation, cleaved by granzyme B, taken up by chemokine receptors on antigen-presenting cells, subsequently enters the processing and presentation pathway, and ultimately elicits an immune response [59, 68]. Thus, autoantigens may act as danger signals to alert the immune response to tissue damage, with dendritic and T cell activation [68]. Supporting this hypothesis, when myositis was induced in a mouse model by overexpression of MHC class I molecules, anti-Jo-1 antibodies developed secondarily [57].

To further assess the role of autoantibodies in myositis, Casciola-Rosen *et al.* [71] examined autoantigen expression in muscle from patients with DM. In lysates from muscle biopsies of patients with DM and controls with histologically normal biopsies, Mi-2, a regulator of nuclear transcription, was expressed at levels 10-fold higher in patients with DM versus control patients. In addition, similar to MHC class I expression, regenerating cells in the perifascicular region in DM muscle expressed high levels of HisRS and cultured myoblasts expressed high levels of Mi-2 and HisRS. Since muscle injury and subsequent myocyte regeneration can be seen in a heterogeneous distribution within muscle, the authors suggest this may explain the sometimes patchy histologic changes in myositis [71]. Mi-2 antibodies have been reported with similar prevalence in different subtypes of myositis, supporting the theory that these are reactive

from inflammation instead of pathogenic in origin [72, 73]. The clinical relevance of Mi-2 antibodies is unknown. An increased rate of malignancy has been associated with antibodies against the N-terminal fragment of Mi-2 β [72]. Others, however, have cited the usefulness of Mi-2 antibody presence in exclusion of paraneoplastic disease [73]. Overall, the data support the view that regenerating muscle cells are the source for a currently unknown autoantigen which may stimulate the immune response in myositis and perpetuate the inflammatory cycle.

The presence of a truly specific antibody which shows transferable pathogenesis would suggest a primary role of the antibody in the pathogenesis of DM. We are unaware of any studies which indicate that serum containing myocyte-specific antibodies is able to induce DM-specific changes and keratinocyte apoptosis. Mi-2 is generally considered DM specific, though a number of studies have reported its presence in inclusion body myositis, PM and other connective tissue diseases [72, 73]. This difference, however, may be attributed to the type of test used to identify the antibodies. In a recent analysis, Ghirardello et al. [73] reported positive Mi-2 antibodies only in the six subjects with DM but not in those with PM or an overlap syndrome when analyzing the tissue specimens with Western blot analysis instead of the previous attempts which used immunodiffusion, immunoprecipitation or ELISA-based techniques. Sato et al. [74] recently reported an amyopathic DM antibody anti-CADM-140. Further studies are required to determine whether this indicates a meaningful difference in the disease cascade among subsets of DM or not.

Endothelial Dysfunction

The vasculopathy present in DM is one of the only ways to differentiate the histologic appearance of skin in DM and CLE [25, 28, 75]. The classic features of endothelial dysfunction in DM include tubuloreticular inclusions, swelling, microvacuolization and necrosis [7, 76]. A decreased capillary index is found in DM patients, even in those without clinically present muscle disease [76]. Interestingly, a recent study of muscle samples in adult DM patients indicates that neovascularization and angiogenesis-specific gene expression may be increased in response to capillary loss [77]. Endothelial cell swelling is one of the earliest pathologic changes seen in the muscle of DM patients, often present before the inflammatory infiltrate can be visualized [10]. The endothelial histologic findings of amyopathic and classic DM are equivalent [28], suggesting that the same underlying mechanisms of disease may be active in both.

Interestingly, gene expression profiles do not show differential regulation of known intramuscular endothelial cell proteins or growth factors [21, 78].

The muscle atrophy seen histologically in DM is sometimes credited to perimysial ischemia following complement-mediated thrombosis and capillary dropout [1]. Considering muscle anatomy and its vascular supply, vessel damage may induce damage in a watershed distribution, leading to preferential myocyte destruction in the perifascicular region and ultimately localized atrophy, although it has been noted that no real evidence exists to support such a claim [7]. However, increased succinate dehydrogenase and reduced cytochrome *c* oxidase enzyme staining, which indicate mitochondrial dysfunction, were identified in 11 of 12 patients with DM, suggesting ischemia as the culprit in perifascicular atrophy [78]. Complement activation and subsequent MAC deposition into the larger vessels may cause muscle ischemia and subsequent myocyte damage [1]. Nagaraju et al. [24] propose an alternative theory for how vasculopathy may indirectly initiate myocyte damage. Ischemia, perhaps secondary to MAC deposition, or any one of a group of potential triggers (such as infection and denervation) may induce myocyte MHC I upregulation and initiate muscle damage via the ER stress response, as mentioned previously. Besides these theories, it has been suggested that endothelial damage may also allow for enhanced perifollicular T cell migration, which in the setting of MHC I upregulation, may promote a cytotoxic T cell response [20, 21].

The evidence supporting the role of ischemia in muscle atrophy of DM is not uncontested, however. Complement activation and deposition, if thought to be the cause of vessel loss and myofiber ischemia, should lead to subsequent complement deposition in the vessel walls. Linking the vasculopathy, ischemia, complement dysfunction and atrophic changes is difficult, however, since complement deposits in perifascicular and endomysial endothelium have been found to be inversely correlated with perifascicular atrophy [76]. Furthermore, there has been no consistent correlation between zones of capillary depletion and perifascicular atrophy [76]. Although ischemia may cause muscle atrophy, whether this is a direct consequence of the complement cascade has been questioned [7]. Alternatively, some have suggested dysregulated type I IFN release may be responsible for perifascicular atrophy [8]. Overall, the relationship between the perimysial vasculopathy and the myocytotoxicity in DM is not well understood.

Endothelial dysfunction in DM is also implicated by data showing the presence of both IL-1 α and ICAM-1 both in muscle samples with [78] and without [21] inflammatory infiltrates. Although endothelial dysfunction seems to play an important role in the pathogenesis of DM, neither the onset nor the downstream events are well understood. Besides the potential relationship between muscle

damage and endothelial dysfunction, anti-endothelial antibodies have also been implicated in the pathogenesis of DM-related ILD. In a study by Cervera et al. [54], examining 18 patients with DM, all 6 patients with ILD had endothelial antibodies in comparison to 2 of 12 without ILD.

Complement, Mannose-Binding Lectin and Clearance

The current dogma regarding the pathogenesis of DM is that it is a humorally mediated autoimmune disease in which antigen-specific antibodies bind to endothelial cells followed by activation of the complement system [2, 7]. As a result of C1 or C3 activation, the C5b-9 MAC is deposited in blood vessel walls, causing capillary necrosis, perivascular inflammation and infiltration of muscle by B cells, resulting in endofascicular hypoperfusion, muscle ischemia and perifascicular atrophy [7, 8]. Complement activation also results in cytokine and chemokine release, which recruits CD4⁺ T cells and macrophages to the affected muscle tissue.

The role of complement in either the onset or the maintenance of DM is unclear, especially in light of a case of DM found in someone with C9 deficiency [79]. MAC deposition has been found surrounding capillaries instead of binding endothelial cell surfaces directly, which challenges the classic model of complement activation in DM [76]. It is still unclear if anti-endothelial antibodies are primarily responsible for activating complement and initiating endothelial damage. In light of discovering complement complexes outside of vessel walls, complement may actually bind to intravascular complexes and undergo subsequent extravascular migration. Kissel et al. [80] have noted the lack of evidence supporting the coaggregation of IgM, C3 and MAC, further questioning the classic model.

Complement deficiencies, which have been reported in SLE [81], are thought to result in decreased clearance of apoptotic bodies and necrotic debris [81], which may lead to prolonged antigen presentation, immune activation and stimulation of cell-mediated host immunity. Interestingly, the complement receptor 2 (CR2) has been shown to play a role in B and T cell tolerance [81]. The appropriate stimulation for CR2-induced tolerance may be lacking in a complement-deficient state [81]. Since similarities in pathogenesis of DM and lupus have been noted [7, 28, 40, 41, 82], it is reasonable to think that impaired clearance of apoptotic bodies through complement in DM may contribute to disease pathogenesis as well. Impaired clearance through deficiencies in mannose-binding lectin has been associated with DM [82]. Despite some of the similarities that exist between DM and LE, they are separate disease entities; recent molecular studies have shown clear differences in HLA genetic frequencies

Table 1. HLA allele associations with DM [58, 83–86]

White	DRB1*0301, DQA1*0501, DQB1*0201 EYSTS, DRB1 HRV motif DRB1*0201 and DR4 (protective)
Hispanic	None detected
Japanese	DRB1*08, DRw59

in DM and LE, highlighting an important and potentially fundamental difference in the two diseases [43].

Genetics

Susceptibility to DM is thought to result from a complex interplay of various gene products. The search for HLA linkage markers has only yielded a few candidate loci for further study [83]. Reed and Ytterberg [83] summarize these HLA associations in a recent review (table 1) [79, 84–86]. Associations between HLA class III and non-HLA immunomodulations IL-1 α , IL-1 β , TNF- α and mannose-binding lectin have been described as well [43, 82, 83].

An association of both HLA-DR3 and HLA-DQ2 [58] and anti-Jo-1 autoantibody has been reported. Tezak et al. [40] have demonstrated a relationship between HLA DQA*0501 and the similar entity JDM.

Environment

Various environmental triggers, including medications, sunlight and infection, have been examined for a relationship to DM [83]. Both atorvastatin and phenytoin have been shown to induce DM, as is true for IFN- α 2b [5, 46]. Both spatial and temporal clustering in DM has been reported [87], suggesting a viral trigger [5, 83]. Various attempts to isolate or identify viral infection, however, have been unsuccessful for a host of pathogens, including coxsackie virus, influenza virus, paramyxovirus, adenovirus, human immunodeficiency virus, human T lymphotropic virus I or II, encephalomyocarditis virus, parvovirus, enterovirus and hepatitis C virus [64, 83]. Testing by seeking out infection with toxoplasmosis and lyme (*Borrelia burgdorfi*) has also turned out negative [83]. Although a consistent infection has not been identified, it is still likely that some not yet identified infection serves as a trigger for a self-sustaining autoimmune response that continues long after elimination of the pathogen. Sunlight is also affected by geography and season, which may explain the spatial and temporal clustering patterns described in DM as well [83].

Relationship to Cancer: Paraneoplastic Phenomena

Patients with DM have been shown to be at increased risk for internal malignancies, independent of the extent of muscle involvement [6, 88]. It has been suggested that the presence of cutaneous necrosis and an erythrocyte sedimentation rate greater than 40 mm/h are significant predictive features to determine which subset of DM patients will ultimately present with a malignancy [3]. Although the process is not well understood, DM occurring in the setting of a primary malignant neoplasm is thought to occur as a paraneoplastic response to the malignancy. Cancers may present self-antigens, including myositis autoantigens, at a greater frequency than is normally seen by the immune system given the monoclonal and rapidly expanding nature of tumors [71, 88]. Nonspecific muscle injury could induce myoblast development and increase the number of regenerating myocytes, which have been shown to express a similar antigen fingerprint as myositis-associated tumors [71]. In conjunction with upregulated MHC I [20, 21, 23], myocyte injury could result from tumor-specific cytotoxic T cells that recognize antigen shared by these regenerating myocytes and tumor cells [71]. Lastly, cancers are known to produce autoantibodies causing a host of paraneoplastic phenomena including Cushing's disease and hypercalcemia [88]. Interestingly, Targoff et al. [60] describe a newly discovered anti-p155 autoantibody which was identified in 6 of 6 subjects with cancer-associated DM but in none of 2 subjects with cancer-associated PM. Internal malignancy may also be associated with another newly described autoantibody which precipitates both 155- and 140-kDa proteins [89]. In the study by Kaji et al. [89], 71% of those with the anti-155/140 antibody were found to have an internal malignancy in comparison to only 11% of those who tested negative for the antibody. Although both the Targoff and Kaji autoantibodies precipitate a 155-kDa protein, they are likely distinct, since the anti-155/140 antibody always coprecipitates a 140-kDa protein, whereas the Targoff anti-155 autoantibody does not [89]. It is unclear, however, whether these autoantibodies represent a host response to a myositis autoantigen or a paraneoplastic phenomenon. Although the direct pathogenesis of autoantibodies in DM is unproven, myositis-associated tumors may provide a stimulus that initiates the host inflammatory reaction.

Relationship to ILD

The association between anti-endothelial antibodies and ILD in DM has already been mentioned. The antibody anti-Jo-1 has also been associated with ILD [64] as part of the antisynthetase syndrome [5]. CD8⁺ lymphocytes were

discovered upon bronchoalveolar lavage of an amyopathic DM patient with refractory ILD, suggesting a role of cytotoxic T cell-mediated lung injury in DM [90]. A recent study comparing the blood serum levels of various profibrotic factors in DM or PM patients showed increased levels of TGF- β , KL-6, ET-1, TM and PAI-1 in those with interstitial pneumonitis [91]. A study in Japanese women with idiopathic inflammatory myopathy showed that HLA class II haplotypes are different in those that do or do not experience interstitial pneumonitis [92]. Furthermore, Sato et al. [74] showed a significant difference in the rapid progression of ILD in amyopathic subjects who had presence of the anti-CADM-140 antibody.

Relationship to JDM

JDM is a chronic autoimmune disorder which primarily affects the skin and muscles of children and young adults [45]. Adult DM and JDM present similarly, except that the calcification in the latter is both more common and severe. Given the common features between these two diseases, lessons learned from studying the pathogenesis of JDM may be useful in understanding the adult disorder. First, like in adult DM, IFN- α , IFN- β and IFN- γ are upregulated in muscle biopsy specimens of JDM [40]. Tezak et al. [40] propose that increased IFN expression in JDM may occur as a postviral, innate immune response. Although good evidence linking either adult or JDM to viral infection is lacking, in light of our current limited understanding of disease pathogenesis it cannot yet be dismissed either. Second, similar to how IFN-responsive gene products are thought to cause muscle ischemia in adult DM, the IFN- γ -responsive genes IP-30 and IP-10, T lymphocyte-specific chemokines, interfere with neo-vascularization, leading to growth arrest of vascular endothelium and capillary dropout [40, 93]. Thus, for both adult DM and JDM the literature supports the role of IFN upregulation, possibly in response to viral infection, a progression to vasculopathy, functional ischemia and myocyte death. Though separate clinical entities, adult DM and JDM share enough in common that advances in understanding either disease may prove helpful in discerning the pathogenesis of the other.

Conclusion

In summary, the literature regarding the pathogenesis and basic science mechanisms of DM presents a disjointed array of seemingly unrelated topics. Although some data clearly outline specific dysfunctional mechanisms in either

the skin or the muscle, oftentimes pathology is found within both tissue types as well as in a systemic distribution. Autoantibody production, endothelial damage, dysregulation of both complement and mannose-binding lectin, and impaired clearance are all pathologic features of DM which are active systemically. Given the complexity of this disease, each of these systems is likely involved in a not yet well understood cascade of disease susceptibility and progression.

References

- 1 Mantegazza R, Bernasconi P, Confalonieri P, Cornelio F: Inflammatory myopathies and systemic disorders: a review of immunopathogenetic mechanisms and clinical features. *J Neurol* 1997;244: 277–287.
- 2 Dalakas MC, Hohlfeld R: Polymyositis and dermatomyositis. *Lancet* 2003;362:971–982.
- 3 Basset-Seguín N, Roujeau JC, Gherardi R, Guillaume JC, Revuz J, Touraine R: Prognostic factors and predictive signs of malignancy in adult dermatomyositis: a study of 32 cases. *Arch Dermatol* 1990;126:633–637.
- 4 Hill CL, Zhang Y, Sigurgeirsson B, Pukkala E, Mellekmjaer L, Airio A, et al: Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. *Lancet* 2001;357:96–100.
- 5 Sontheimer RD: Dermatomyositis: an overview of recent progress with emphasis on dermatologic aspects. *Dermatol Clin* 2002;20:387–408.
- 6 Gerami P, Schope JM, McDonald L, Walling HW, Sontheimer RD: A systematic review of adult-onset clinically amyopathic dermatomyositis (dermatomyositis sine myositis): a missing link within the spectrum of the idiopathic inflammatory myopathies. *J Am Acad Dermatol* 2006;54: 597–613.
- 7 Greenberg SA, Amato AA: Uncertainties in the pathogenesis of adult dermatomyositis. *Curr Opin Neurol* 2004;17:359–364.
- 8 Greenberg SA, Pinkus JL, Pinkus GS, Burleson T, Sanoudou D, Tawil R, et al: Interferon- α/β -mediated innate immune mechanisms in dermatomyositis. *Ann Neurol* 2005;57:664–678.
- 9 Greenberg SAM, Sanoudou DP, Haslett JN, Kohane ISMDP, Kunkel LMP, Beggs AHP, et al: Molecular profiles of inflammatory myopathies. *Neurology* 2002;59:1170–1182.
- 10 Dalakas MC: Muscle biopsy findings in inflammatory myopathies. *Rheum Dis Clin North Am* 2002;28:779–798, vi.
- 11 Naik SH, Corcoran LM, Wu L: Development of murine plasmacytoid dendritic cell subsets. *Immunol Cell Biol* 2005;83:563–570.
- 12 Page G, Chevrel G, Miossec P: Anatomic localization of immature and mature dendritic cell subsets in dermatomyositis and polymyositis: interaction with chemokines and Th1 cytokine-producing cells. *Arthritis Rheum* 2004;50:199–208.
- 13 Bank I, Miranda AF, Chess L: Mechanisms of cell-mediated myocytotoxicity in the peripheral blood of patients with inflammatory myopathies. *J Clin Immunol* 2001;21:328–334.
- 14 Goebels N, Michaelis D, Engelhardt M, Huber S, Bender A, Pongratz D, et al: Differential expression of perforin in muscle-infiltrating T cells in polymyositis and dermatomyositis. *J Clin Invest* 1996;97:2905–2910.
- 15 Figarella-Branger D, Civatte M, Bartoli C, Pellissier JF: Cytokines, chemokines, and cell adhesion molecules in inflammatory myopathies. *Muscle Nerve* 2003;28:659–682.
- 16 De Bleecker JL, De Paep B, Vanwalleghem IE, Schroder JM: Differential expression of chemokines in inflammatory myopathies. *Neurology* 2002;58:1779–1785.
- 17 Chinoy H, Salway F, Fertig N, Tait BD, Oddis CV, Ollier WE, et al: Monocyte chemotactic protein-1 single nucleotide polymorphisms do not confer susceptibility for the development of adult onset polymyositis/dermatomyositis in UK Caucasians. *Rheumatology (Oxford)* 2007;46: 604–607.

- 18 De Paepe B, De Keyser K, Martin JJ, De Bleecker JL: α -Chemokine receptors CXCR1–3 and their ligands in idiopathic inflammatory myopathies. *Acta Neuropathol (Berl)* 2005;109:576–582.
- 19 Sallum AM, Kiss MH, Silva CA, Wakamatsu A, Vianna MA, Sachetti S, et al: Difference in adhesion molecule expression (ICAM-1 and VCAM-1) in juvenile and adult dermatomyositis, polymyositis and inclusion body myositis. *Autoimmun Rev* 2006;5:93–100.
- 20 Karpati G, Pouliot Y, Carpenter S: Expression of immunoreactive major histocompatibility complex products in human skeletal muscles. *Ann Neurol* 1988;23:64–72.
- 21 Pavlath GK: Regulation of class I MHC expression in skeletal muscle: deleterious effect of aberrant expression on myogenesis. *J Neuroimmunol* 2002;125:42–50.
- 22 Englund P, Nennesmo I, Klareskog L, Lundberg IE: Interleukin-1 α expression in capillaries and major histocompatibility complex class I expression in type II muscle fibers from polymyositis and dermatomyositis patients: important pathogenic features independent of inflammatory cell clusters in muscle tissue. *Arthritis Rheum* 2002;46:1044–1055.
- 23 Nagaraju K, Raben N, Loeffler L, Parker T, Rochon PJ, Lee E, et al: Conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis-specific autoantibodies. *Proc Natl Acad Sci USA* 2000;97:9209–9214.
- 24 Nagaraju K, Casciola-Rosen L, Lundberg I, Rawat R, Cutting S, Thapliyal R, et al: Activation of the endoplasmic reticulum stress response in autoimmune myositis: potential role in muscle fiber damage and dysfunction. *Arthritis Rheum* 2005;52:1824–1835.
- 25 Dourmishev LA, Wollina U: Dermatomyositis: immunopathologic study of skin lesions. *Acta Dermatovenerol Alp Panonica Adriat* 2006;15:45–51.
- 26 Igarashi M, Aizawa H, Tokudome Y, Tagami H: Dermatomyositis with prominent mucinous skin change: histochemical and biochemical aspects of glycosaminoglycans. *Dermatologica* 1985;170:6–11.
- 27 Pablos JL, Santiago B, Galindo M, Carreira PE, Ballestin C, Gomez-Reino JJ: Keratinocyte apoptosis and p53 expression in cutaneous lupus and dermatomyositis. *J Pathol* 1999;188:63–68.
- 28 Crowson AN, Magro CM: The role of microvascular injury in the pathogenesis of cutaneous lesions of dermatomyositis. *Hum Pathol* 1996;27:15–19.
- 29 Caproni M, Torchia D, Cardinali C, Volpi W, Del Bianco E, D’Agata A, et al: Infiltrating cells, related cytokines and chemokine receptors in lesional skin of patients with dermatomyositis. *Br J Dermatol* 2004;151:784–791.
- 30 Wenzel J, Schmidt R, Proelss J, Zahn S, Bieber T, Tuting T: Type I interferon-associated skin recruitment of CXCR3+ lymphocytes in dermatomyositis. *Clin Exp Dermatol* 2006;31:576–582.
- 31 Hausmann G, Herrero C, Cid MC, Casademont J, Lecha M, Mascaro JM: Immunopathologic study of skin lesions in dermatomyositis. *J Am Acad Dermatol* 1991;25:225–230.
- 32 Adams EM, Kirkley J, Eidelman G, Dohlman J, Plotz PH: The predominance of β (CC) chemokine transcripts in idiopathic inflammatory muscle diseases. *Proc Assoc Am Physicians* 1997;109:275–285.
- 33 Raj D, Brash DE, Grossman D: Keratinocyte apoptosis in epidermal development and disease. *J Invest Dermatol* 2006;126:243–257.
- 34 Chung JH, Kwon OS, Eun HC, Youn JI, Song YW, Kim JG, et al: Apoptosis in the pathogenesis of cutaneous lupus erythematosus. *Am J Dermatopathol* 1998;20:233–241.
- 35 Kuhn A, Herrmann M, Kleber S, Beckmann-Welle M, Fehsel K, Martin-Villalba A, et al: Accumulation of apoptotic cells in the epidermis of patients with cutaneous lupus erythematosus after ultraviolet irradiation. *Arthritis Rheum* 2006;54:939–950.
- 36 Furukawa F, Kashiwara-Sawami M, Lyons MB, Norris DA: Binding of antibodies to the extractable nuclear antigens SS-A/Ro and SS-B/La is induced on the surface of human keratinocytes by ultraviolet light (UVL): implications for the pathogenesis of photosensitive cutaneous lupus. *J Invest Dermatol* 1990;94:77–85.
- 37 Jones SK: Ultraviolet radiation (UVR) induces cell-surface Ro/SSA antigen expression by human keratinocytes in vitro: a possible mechanism for the UVR induction of cutaneous lupus lesions. *Br J Dermatol* 1992;126:546–553.
- 38 Golan TD, Elkon KB, Gharavi AE, Krueger JG: Enhanced membrane binding of autoantibodies to cultured keratinocytes of systemic lupus erythematosus patients after ultraviolet B/ultraviolet A irradiation. *J Clin Invest* 1992;90:1067–1076.

- 39 Kawashima T, Zappi EG, Lieu TS, Sontheimer RD: Impact of ultraviolet irradiation on expression of SSA/Ro autoantigenic polypeptides in transformed human epidermal keratinocytes. *Lupus* 1994;3:493–500.
- 40 Tezak Z, Hoffman EP, Lutz JL, Fedczyna TO, Stephan D, Bremer EG, et al: Gene expression profiling in DQA1*0501+ children with untreated dermatomyositis: a novel model of pathogenesis. *J Immunol* 2002;168:4154–4163.
- 41 Magro CM, Crowson AN: The immunofluorescent profile of dermatomyositis: a comparative study with lupus erythematosus. *J Cutan Pathol* 1997;24:543–552.
- 42 Pupe A, Degreef H, Garmyn M: Induction of tumor necrosis factor- α by UVB: a role for reactive oxygen intermediates and eicosanoids. *Photochem Photobiol* 2003;78:68–74.
- 43 Werth VP, Callen JP, Ang G, Sullivan KE: Associations of tumor necrosis factor α and HLA polymorphisms with adult dermatomyositis: implications for a unique pathogenesis. *J Invest Dermatol* 2002;119:617–620.
- 44 Werth VP, Zhang W, Dortzbach K, Sullivan K: Association of a promoter polymorphism of tumor necrosis factor- α with subacute cutaneous lupus erythematosus and distinct photoregulation of transcription. *J Invest Dermatol* 2000;115:726–730.
- 45 Pachman LM: Juvenile dermatomyositis: immunogenetics, pathophysiology, and disease expression. *Rheum Dis Clin North Am* 2002;28:579–602, vii.
- 46 Dietrich LL, Bridges AJ, Albertini MR: Dermatomyositis after interferon α treatment. *Med Oncol* 2000;17:64–69.
- 47 Janeway C: *Immunobiology: The Immune System in Health and Disease*, ed 6. New York, Garland Science, 2005.
- 48 Del Pozo J, Almagro M, Martinez W, Yebra-Pimentel MT, Garcia-Silva J, Pena-Penabad C, et al: Dermatomyositis and mucinosis. *Int J Dermatol* 2001;40:120–124.
- 49 Johnson BL, Horowitz IR, Charles CR, Cooper DL: Dermatomyositis and lichen myxedematosus: a clinical, histopathological and electron microscopic study. *Dermatologica* 1973;147:109–122.
- 50 Requena L, Aguilar A, Sanchez Yus E: A corrugated plaque on the abdominal wall: cutaneous mucinosis secondary to dermatomyositis. *Arch Dermatol* 1990;126:1639–1640, 1642–1643.
- 51 Falanga V, Tiesgs SL, Alstadt SP, Roberts AB, Sporn MB: Transforming growth factor- β : selective increase in glycosaminoglycan synthesis by cultures of fibroblasts from patients with progressive systemic sclerosis. *J Invest Dermatol* 1987;89:100–104.
- 52 Postlethwaite AE, Smith GN Jr, Lachman LB, Endres RO, Poppleton HM, Hasty KA, et al: Stimulation of glycosaminoglycan synthesis in cultured human dermal fibroblasts by interleukin 1: induction of hyaluronic acid synthesis by natural and recombinant interleukin 1s and synthetic interleukin 1 β peptide 163–171. *J Clin Invest* 1989;83:629–636.
- 53 Duncan MR, Berman B: Stimulation of collagen and glycosaminoglycan production in cultured human adult dermal fibroblasts by recombinant human interleukin 6. *J Invest Dermatol* 1991;97:686–692.
- 54 Cervera R, Ramirez G, Fernandez-Sola J, D'Cruz D, Casademont J, Grau JM, et al: Antibodies to endothelial cells in dermatomyositis: association with interstitial lung disease. *BMJ* 1991;302:880–881.
- 55 Whitaker JN, Engel WK: Vascular deposits of immunoglobulin and complement in idiopathic inflammatory myopathy. *N Engl J Med* 1972;286:333–338.
- 56 Miller FW: Myositis-specific autoantibodies: touchstones for understanding the inflammatory myopathies. *JAMA* 1993;270:1846–1849.
- 57 Targoff IN: Update on myositis-specific and myositis-associated autoantibodies. *Curr Opin Rheumatol* 2000;12:475–481.
- 58 Love LA, Leff RL, Fraser DD, Targoff IN, Dalakas M, Plotz PH, et al: A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. *Medicine (Baltimore)* 1991;70:360–374.
- 59 Howard OMZ, Dong HF, Yang D, Raben N, Nagaraju K, Rosen A, et al: Histidyl-tRNA synthetase and asparaginyl-tRNA synthetase, autoantigens in myositis, activate chemokine receptors on T lymphocytes and immature dendritic cells. *J Exp Med* 2002;196:781–791.
- 60 Targoff IN, Mamuyrova G, Trieu EP, Perurena O, Koneru B, O'Hanlon TP, et al: A novel autoantibody to a 155-kD protein is associated with dermatomyositis. *Arthritis Rheum* 2006;54:3682–3689.

- 61 Levine SM, Rosen A, Casciola-Rosen LA: Anti-aminoacyl tRNA synthetase immune responses: insights into the pathogenesis of the idiopathic inflammatory myopathies. *Curr Opin Rheumatol* 2003;15:708–713.
- 62 Kang EH, Lee EB, Shin KC, Im CH, Chung DH, Han SK, et al: Interstitial lung disease in patients with polymyositis, dermatomyositis and amyopathic dermatomyositis. *Rheumatology (Oxford)* 2005;44:1282–1286.
- 63 Ioannou Y, Sultan S, Isenberg DA: Myositis overlap syndromes. *Curr Opin Rheumatol* 1999;11: 468–474.
- 64 Dalakas MC: Immunopathogenesis of inflammatory myopathies. *Ann Neurol* 1995;37(suppl 1): S74–S86.
- 65 Marie I, Hachulla E, Cherin P, Dominique S, Hatron PY, Hellot MF, et al: Interstitial lung disease in polymyositis and dermatomyositis. *Arthritis Rheum* 2002;47:614–622.
- 66 Mozaffar T, Pestronk A: Myopathy with anti-Jo-1 antibodies: pathology in perimysium and neighbouring muscle fibres. *J Neurol Neurosurg Psychiatry* 2000;68:472–478.
- 67 Blechynden LM, Lawson MA, Tabarias H, Garlepp MJ, Sherman J, Raben N, et al: Myositis induced by naked DNA immunization with the gene for histidyl-tRNA synthetase. *Hum Gene Ther* 1997;8:1469–1480.
- 68 Plotz PH: The autoantibody repertoire: searching for order. *Nat Rev Immunol* 2003;3:73–78.
- 69 Oppenheim JJ, Dong HF, Plotz P, Caspi RR, Dykstra M, Pierce S, et al: Autoantigens act as tissue-specific chemoattractants. *J Leukoc Biol* 2005;77:854–861.
- 70 Casciola-Rosen L, Andrade F, Ulanet D, Wong WB, Rosen A: Cleavage by granzyme B is strongly predictive of autoantigen status: implications for initiation of autoimmunity. *J Exp Med* 1999;190: 815–826.
- 71 Casciola-Rosen L, Nagaraju K, Plotz P, Wang K, Levine S, Gabrielson E, et al: Enhanced autoantigen expression in regenerating muscle cells in idiopathic inflammatory myopathy. *J Exp Med* 2005;201:591–601.
- 72 Hengstman GJ, Vree Egberts WT, Seelig HP, Lundberg IE, Moutsopoulos HM, Doria A, et al: Clinical characteristics of patients with myositis and autoantibodies to different fragments of the Mi-2 β antigen. *Ann Rheum Dis* 2006;65:242–245.
- 73 Ghirardello A, Zampieri S, Iaccarino L, Tarricone E, Bendo R, Gambari PF, et al: Anti-Mi-2 antibodies. *Autoimmunity* 2005;38:79–83.
- 74 Sato S, Hirakata M, Kuwana M, Suwa A, Inada S, Mimori T, et al: Autoantibodies to a 140-kD polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. *Arthritis Rheum* 2005;52:1571–1576.
- 75 Mascaro JM Jr, Hausmann G, Herrero C, Grau JM, Cid MC, Palou J, et al: Membrane attack complex deposits in cutaneous lesions of dermatomyositis. *Arch Dermatol* 1995;131: 1386–1392.
- 76 Emslie-Smith AM, Engel AG: Microvascular changes in early and advanced dermatomyositis: a quantitative study. *Ann Neurol* 1990;27:343–356.
- 77 Nagaraju K, Rider LG, Fan C, Chen YW, Mitsak M, Rawat R, et al: Endothelial cell activation and neovascularization are prominent in dermatomyositis. *J Autoimmune Dis* 2006;3:2.
- 78 Alhatou MI, Sladky JT, Bagasra O, Glass JD: Mitochondrial abnormalities in dermatomyositis: characteristic pattern of neuropathology. *J Mol Histol* 2004;35:615–619.
- 79 Ichikawa E, Furuta J, Kawachi Y, Imakado S, Otsuka F: Hereditary complement (C9) deficiency associated with dermatomyositis. 2001;144:1080–1083.
- 80 Kissel JT, Mendell JR, Rammohan KW: Microvascular deposition of complement membrane attack complex in dermatomyositis. *N Engl J Med* 1986;314:329–334.
- 81 Boackle SA: Complement and autoimmunity. *Biomed Pharmacother* 2003;57:269–273.
- 82 Werth VP, Berlin JA, Callen JP, Mick R, Sullivan KE: Mannose binding lectin (MBL) polymorphisms associated with low MBL production in patients with dermatomyositis. *J Invest Dermatol* 2002;119:1394–1399.
- 83 Reed AM, Ytterberg SR: Genetic and environmental risk factors for idiopathic inflammatory myopathies. *Rheum Dis Clin North Am* 2002;28:891–916.
- 84 Arnett FC: HLA genes and predisposition to rheumatic diseases. *Hosp Pract (Off Ed)* 1986;21: 89–100.

- 85 Hirsch TJ, Enlow RW, Bias WB, Arnett FC: HLA-D related (DR) antigens in various kinds of myositis. *Hum Immunol* 1981;3:181–186.
- 86 Furuya T, Hakoda M, Higami K, Ueda H, Tsuchiya N, Tokunaga K, et al: Association of HLA class I and class II alleles with myositis in Japanese patients. *J Rheumatol* 1998;25:1109–1114.
- 87 Rosenberg A: Geographic clustering of childhood dermatomyositis in Saskatchewan. *Arthritis Rheum* 1994;37(suppl 9):S402.
- 88 Marmur R, Kagen L: Cancer-associated neuromusculoskeletal syndromes; recognizing the rheumatic-neoplastic connection. *Postgrad Med* 2002;111:95–98, 101–102.
- 89 Kaji K, Fujimoto M, Hasegawa M, Kondo M, Saito Y, Komura K, et al: Identification of a novel autoantibody reactive with 155 and 140 kDa nuclear proteins in patients with dermatomyositis: an association with malignancy. *Rheumatology (Oxford)* 2007;46:25–28.
- 90 Yokoyama T, Sakamoto T, Shida N, Kinoshita M, Kunou Y, Karukaya R, et al: Fatal rapidly progressive interstitial pneumonitis associated with amyopathic dermatomyositis and CD8 T lymphocytes. *J Intensive Care Med* 2005;20:160–163.
- 91 Funauchi M, Shimadzu H, Tamaki C, Yamagata T, Nozaki Y, Sugiyama M, et al: Role of endothelial damage in the pathogenesis of interstitial pneumonitis in patients with polymyositis and dermatomyositis. *J Rheumatol* 2006;33:903–906.
- 92 Horiki T, Ichikawa Y, Moriuchi J, Hoshina Y, Yamada C, Wakabayashi T, et al: HLA class II haplotypes associated with pulmonary interstitial lesions of polymyositis/dermatomyositis in Japanese patients. *Tissue Antigens* 2002;59:25–30.
- 93 Fall N, Bove KE, Stringer K, Lovell DJ, Brunner HI, Weiss J, et al: Association between lack of angiogenic response in muscle tissue and high expression of angiostatic ELR-negative CXC chemokines in patients with juvenile dermatomyositis: possible link to vasculopathy. *Arthritis Rheum* 2005;52:3175–3180.

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Novel Mechanism for Therapeutic Action of IVIg in Autoimmune Blistering Dermatoses

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Abstract

The mode of action of intravenous immunoglobulin (IVIg) is complex. An ongoing research continues to elaborate and identify novel mechanisms. Recent advances have demonstrated that IVIg has direct effect on keratinocytes, the target cells of autoimmune blistering diseases. IVIg protects keratinocytes from pathogenic autoantibodies by preventing the autoantibody-induced of apoptosis and oncosis. This anti-apoptotic action of IVIg helps explain how IVIg works in severe, life threatening dermatologic conditions that are resistant to traditional systemic treatments, such as toxic epidermal necrolysis and Stevens-Johnson syndrome. Thus, the actions of IVIg are varied and complex, and the primary mechanisms of action may be different in different diseases.

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Once reserved for treatment of immune deficiencies, intravenous immunoglobulin (IVIg) has found use in a variety of autoimmune diseases. The mechanisms of action of IVIg in autoimmune diseases is complex and research continues to elaborate and identify novel mechanisms. One area in which IVIg is continuing to make dramatic improvements in our ability to treat derangements of immunity is in autoimmune blistering disorders such as pemphigus vulgaris, pemphigus foliaceus, epidermolysis bullosa aquisita, mucous membrane pemphigoid and bullous pemphigoid [1, 2]. Recent advances have demonstrated that IVIg has direct as well as indirect effects on keratinocytes [3].

IVIg is primarily IgG concentrated from blood pooled from thousands (usually 3,000–10,000) of presumed healthy donors. Initially, IVIg was used to treat

Table 1. Possible mechanisms of therapeutic action of IVIg in autoimmune blistering dermatoses

Alterations with effector cells

Antiproliferative effect on T cells [47]
Induction of apoptosis in lymphocytes and monocytes [48, 49]
Synergistic suppression of lymphocyte activation by corticosteroids [50]
Regulation of cytokine secretion [51, 52]
Anti-cytokine antibodies [49]
Inhibition of Fc receptor-mediated phagocytosis [53]
Fc receptor-mediated inhibition of antibody production [14]
Immunoregulatory substances CD4, HLA and TGF- β [30]
Upregulation of Bcl-2 expression [44]
Inhibition of TNF- α signaling pathways [53, 54]

Inhibition of humoral effectors

Natural autoantibodies/anti-idiotypic network [17]
Inhibition of complement membrane attack complex [55]
Inactivation of active Fas ligand in patients' serum [56]

Protection of target cells

Inhibition of keratinocytes apoptosis in combination with high-dose dexamethasone [57]
Inhibition of Fas-mediated apoptosis of keratinocytes [56]
Upregulation of antiapoptotic FLIP and antioncotic calpastatin in keratinocytes [3]

immune deficiencies, but was subsequently found to be effective in idiopathic thrombocytopenic purpura [4] and other autoimmune diseases, including autoimmune blistering diseases. In addition to IgG, IVIg contains lesser amounts of other immunoglobulins (primarily IgM and IgA) and soluble immunologically active components such as cytokines, CD4, CD8 and HLA molecules [5]. IVIg exerts disparate effects through a multiplicity of actions, many of which are still being elucidated. Some of these actions are listed in table 1.

Pathogenic autoantibodies that mediate much of the disease pathology are a common feature in autoimmune bullous diseases. These antibodies bind to keratinocytes and other skin components to produce cell death and blister formation. There is evidence that the autoantibodies induce keratinocyte death through at least two mechanisms, apoptosis and oncosis [3]. Through its various mechanisms, IVIg effectively reduces or eliminates the effects of autoantibodies. Interestingly, in addition to previously elucidated immunomodulatory mechanisms, recent evidence suggests that IVIg exerts a protective effect directly on the keratinocytes by protecting them from pathogenic autoantibodies and preventing the induction of apoptosis and oncosis [3]. We will review

some of the classic mechanisms through which IVIg acts in autoimmune blistering diseases and introduce the novel mechanisms of protection that have recently been discovered.

Clinical Efficacy of IVIg in Autoimmune Blistering Diseases

IVIg has been shown to be helpful for autoimmune blistering diseases that are resistant to traditional systemic immunosuppression [6]. In such extreme cases, it was shown to achieve control of disease and reduce the use of systemic steroids by an average of 41% within 3 weeks [6]. In another study, 21 patients with pemphigus vulgaris unresponsive to systemic steroids were treated with IVIg. With treatment courses averaging 27–30 months, all patients were able to taper off systemic steroids and achieve remission that lasted until after the study ended (13–73 months) [7].

A similar trial was able to induce remission in all 11 patients for an average of 18.6 months of observation [8]. A review of the literature on IVIg use for bullous pemphigoid ranging from one dose to repeated doses at 2 mg/kg/cycle showed that 12 (70%) of 17 patients achieved a good clinical response [1]. A single series of 15 patients with severe recurrent bullous pemphigoid demonstrated that all patients were able to stop use of other medications and remained in remission for more than 3 years after treatment with IVIg.

IVIg has also proven effective in mucous membrane pemphigoid and epidermolysis bullosa acquisita. Multiple studies have demonstrated that in severe mucous membrane pemphigoid, IVIg is more effective at resolving present lesions, preventing progression to additional mucosal sites, and produces longer remissions than conventional immunosuppressive therapy [1]. Similar results were seen in smaller numbers of epidermolysis bullosa acquisita patients [1].

Mechanisms of Therapeutic Action of IVIg

Conventional Explanations

Antibody Depletion

One of the primary pathogenic mechanisms of autoimmune blistering disorders is the action of pathogenic autoantibodies on keratinocytes. A dramatic and rapid effect of treatment with IVIg is the rapid selective decrease in autoimmune antibodies leaving normal protective antibodies unaffected [6, 9, 10]. This correlates well with the rapid therapeutic effect it often has. The rapid decrease suggests that, at least initially, it is due to increased catabolism of

antibody rather than decreased production [11]. This is also supported by evidence that the primary mechanism by which homeostasis of total immunoglobulin levels is maintained is through catabolism, rather than production [12, 13]. Though the exact mechanism remains to be elucidated, studies have suggested that increased IgG may saturate degradation-prevention mechanisms such as the Fc receptor [14].

Direct and Indirect Anti-Idiotypic Effects

The normal human antibody repertoire contains many relatively low-avidity and low-specificity autoreactive antibodies called natural autoantibodies [15, 16]. Most natural autoantibodies are IgG, suggesting an autoreactive T cell repertoire is also involved [17]. Within this natural autoantibody population are antibodies that bind to the variable region of other antibodies. It has been proposed that this anti-idiotypic interaction interferes with autoantibody binding to autoantigen, preventing its effector function. It may also cause anti-idiotypic cross-linking of surface IgG without helper T cell help leading to apoptosis of the autoimmune B cells [18, 19]. Anti-idiotypic antipathogenic autoantibodies have been suggested to be most relevant in antibody-mediated autoimmune neuropathies [20]. Though this does not explain the rapid decrease in autoantibody titer and the selective action against the pathogenic autoimmune antibodies, it would account for the long-term beneficial effects after treatment has stopped.

Antibody-Mediated Effects

There has also been evidence that the antibodies in pooled IVIg may interact with pathogenic autoantibodies. This occurs through blocking the function of the antibodies or by inhibiting binding to autoantigens. This has been demonstrated in *in vitro* studies for antibodies against a number of autoantigens, including factor VIII, DNA, neutrophil cytoplasmic antigens, and acetylcholine receptors [21].

Anti-Inflammatory Effects

In addition to directly reducing autoantibody concentration, IVIg has been shown to exhibit a diverse range of additional effects. Derangement of normal cytokine profiles has been described in pemphigus vulgaris patients for a number of cytokines [22–25]. Selective regulation of cytokine levels by IVIg has been demonstrated [26–28]. For example, in pemphigus vulgaris patients, IL-1 levels are increased and IL-1 receptor antagonist levels are decreased, but after treatment with IVIg they return to levels comparable to those in controls [29]. In some diseases, the major effect of IVIg modulation of cytokines may be regulation of helper T cell subset Th1 and Th2 cytokines [30].

Fc γ Receptor-Mediated Effects

There are receptors on phagocytes that bind to the constant region of IgG antibodies (Fc γ R). These may be activating or inhibitory. By temporarily increasing the concentration of IgG, these receptors may be saturated, preventing their cross-linking activation. In addition, there is evidence that IVIg increases the expression of an inhibitory Fc γ R (Fc γ RIIB). Experimentally, this resulted in decreased clearance of opsonized circulating red blood cells [31] and, theoretically, could act to decrease activation of antikeratinocyte-primed phagocytes.

Dendritic Cell Activation

The effect of IVIg on dendritic cells is multifaceted. When used at lower, replacement levels for immunodeficiencies, it appears to activate differentiation of dendritic cells [32]. At the levels used in treating autoimmune diseases, IVIg blocks maturation of dendritic cells in vitro, resulting in increased IL-10 secretion and decreased IL-12 elaboration [33].

Novel Mechanisms of Target Cell Death and Therapeutic

Action of IVIg in Autoimmune Blistering Dermatoses

All of the mechanisms discussed above primarily deal with the effect of IVIg on circulating antibodies, lymphocytes or antigen-presenting cells. Many of these mechanisms undoubtedly play important roles in the effect of IVIg. In addition, IVIg also appears to act directly in the skin to prevent autoantibody-induced cell death. Deposition of pathogenic autoantibodies has been demonstrated and is, indeed, likely to be intrinsic to the pathology of autoimmune blistering diseases. It has been demonstrated on human keratinocytes in vitro and in mouse models in vivo that antibodies from pemphigus patients induce cell death through two major mechanisms, apoptosis and oncosis, and that IVIg can inhibit both of these pathways [3]. Complementing this, it has been shown in toxic epidermal necrolysis patients that the patients' antibodies are present in the skin and the skin blisters, and that administered IVIg also gets distributed to the skin and skin blisters [34].

Apoptosis, derived from the ancient Greek word referring to the 'falling away' of dying leaves from a tree [35], is a type of programmed cell death and a well orchestrated process in which a cell that is destined to die undergoes energy-dependent well-controlled self destruction or autolysis [35]. It involves specific changes of the nucleus, cytoplasm and cell membrane. Initially, the cell contracts, forming a more spherical profile, and loses contact with its neighbors. In the nucleus, the chromatin condenses into aggregates and the DNA is broken into fragments at regular intervals. The nuclear surface becomes undulated and separates into blebs. The cell membrane also becomes convoluted,

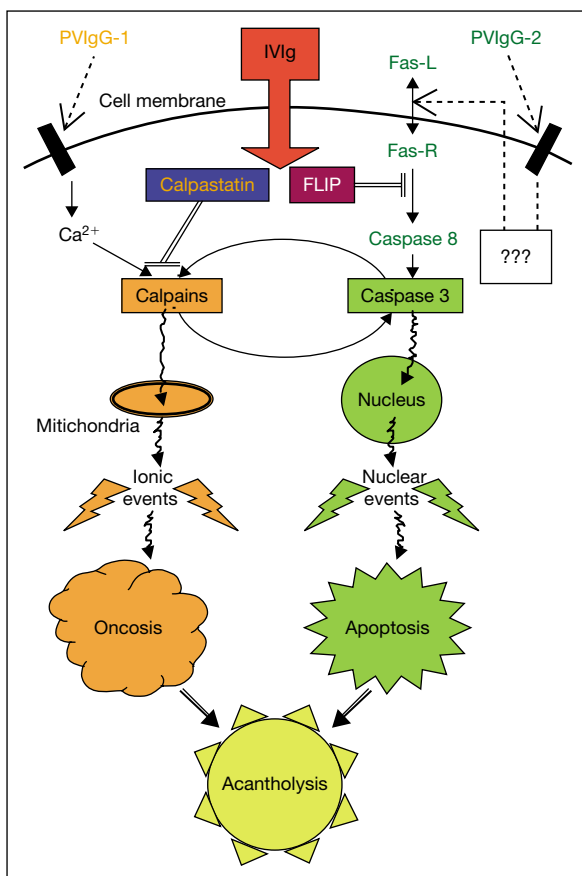


Fig. 1. Hypothetical scheme of the therapeutic action of IVIg in pemphigus. Fas-R = Fas receptor. PVlgG = pemphigus vulgaris autoantibody type 1 or 2. Modified from Arredondo et al. [3].

forming blebs that encapsulate nuclear particles and cytoplasmic contents, and then falls away from the imploding cell [35]. The encapsulating process prevents the uncontrolled release of cellular contents, protecting surrounding tissues from the lytically active intracellular proteins and preventing nonspecific inflammation.

Apoptosis can be induced by a variety of factors from cell-specific (for example Fas-L) to nonspecific signals (for example nitric oxide) that can activate the caspase cascade and ultimately cause cell death (fig. 1). Regulation of cell sensitivity to proapoptotic signals is carried out at multiple levels. The amount of

apoptosis-inducing cell-surface receptors (for example to Fas-L and TNF- α) is controlled by the environment in which a cell exists and by coreceptor signaling [35]. Regulation of the signaling cascade within the cell is also regulated by antiapoptotic proteins (for example Bcl-2 and FLIP) that can inhibit the initiation or amplification of the caspase cascade. FLIP, in particular, is a dominant-negative version of caspase 8 and prevents the formation of the initial death-inducing signaling complex (DISC) that forms when Fas is activated.

In contrast to apoptosis, necrosis (derived from the Greek word for death) is the process of uncontrolled cell death, usually as the result of an overwhelming injury, whose speed or magnitude overcomes the cell's ability to regulate its demise. The cell death is poorly controlled, resulting in the release of cell contents, including the degradative enzymes from lysosomes and the majority of immunogenic molecules from within the cell. This often causes the death or injury of nearby cells and nonspecific inflammation.

A third type of cell death, oncosis, was originally categorized as a type of necrosis [36]. Oncosis is derived from the Greek word for swelling and was thought to involve uncontrolled swelling that caused the cell to burst. As in traditional necrosis, oncosis ends in the release of cell constituents, producing inflammation [37]. A hallmark of oncosis is that, in contrast to apoptosis, it is an energy-independent process often caused by ATP depletion [36]. In addition, there is a loss of volume control resulting in swelling of the nucleus, Golgi apparatus, endoplasmic reticulum as well as the cell as a whole. Specific signaling events are still being worked out and there are probably multiple pathways that produce oncosis, but it has been demonstrated to have a number of regulated stages, including activation of calpains, that likely represent a common final pathway [38]. Like apoptosis, oncosis has intracellular regulators of the enzymatic cascade leading to cell death. Calpastatin acts as an inhibitor of calpain activation. It is normally active, but levels drop during oncosis. Oncosis, therefore, represents a type of controlled cell death distinct from apoptosis, but both eventuate in necrosis.

The etiology and pathogenesis of autoimmune bullous disorders continue to be elucidated. One of the most extensively investigated of these diseases is pemphigus vulgaris, in which a variety of pathogenic IgG autoantibodies are produced that initiate and exacerbate the disease. The blistering that is a hallmark of the disease is a result of the process of acantholysis in which keratinocytes detach from each other and undergo programmed cell death. The autoantibodies produced in pemphigus patients have been demonstrated to increase the levels of, and activate and/or synergize with the proapoptotic Fas and Fas-L [3, 39–41], caspase 1, 3, 5 and 8 [41], Bax, TNF- α [42], c-Myc [43] and nitric oxide. Top candidates for the acantholysis-inducing autoantibodies are antibodies against desmogleins and acetylcholine receptors [45].

Analysis of the activity of enzymes involved primarily in apoptotic or oncotic pathways from keratinocytes treated with IgG antibodies from patients with active pemphigus vulgaris was performed [3]. It demonstrated that the patients could be grouped according to whether their antibodies activated apoptosis-inducing caspase 3 and caspase 8 better than oncosis-inducing calpain, or vice versa. These results indicate that the pathogenic antibodies can activate both pathways separately and help to account for the variability in clinical presentation and response to treatment of patients with pemphigus vulgaris. Having demonstrated that pemphigus vulgaris induces acantholysis through both apoptotic and oncotic pathways, it provides a profitable model to explore the ability of IVIg to act on those pathways in autoimmune blistering diseases.

IVIg prevented the pemphigus vulgaris autoantibody-induced increase in pro-apoptotic caspases, while increasing the expression of the protective FLIP [3]. In addition, IVIg blocked autoantibody-induced increase in calpain activity and upregulated calpastatin expression [3]. These changes correlated with decreased induction of acantholysis *in vitro* in keratinocyte cultures and *in vivo* in the mouse model of experimental pemphigus. Apparently, IVIg protected from apoptosis and oncosis before intercellular adhesion would be lost due to autoantibody action. Already after 12 h of exposure to autoantibodies, apoptosis had started, as evidenced by the fact that mRNA of the proapoptotic proteins caspase 3, caspase 8 and Fas-L were elevated [3]. In contrast, the first gross signs of acantholysis take 24–48 h to occur [46]. These findings demonstrated that IVIg inhibits apoptosis and oncosis in keratinocytes through its direct actions on keratinocytes.

Conclusion

The actions of IVIg are varied and complex. The primary mechanisms of action are different in different diseases. In autoimmune blistering disorders, the production of pathogenic autoantibodies that target self-antigens in keratinocytes is a key etiologic element that is regulated by IVIg and may help to account for therapeutic effects of IVIg. The pathogenic antibodies activate apoptotic and oncotic pathways that lead to the death of keratinocytes and loss of integrity of the epidermis. IVIg acts to block these programmed cell death pathways, preserving keratinocytes and preventing cell-cell and cell-matrix detachments. The protective role of IVIg on keratinocytes opens additional avenues of research to improve our understanding of autoimmune blistering dermatoses and also provides additional therapeutic targets for new treatments. As our understanding of the effects of autoimmune antibodies on the skin grows, so does our understanding of the therapeutic effects of IVIg.

References

- 1 Ahmed AR: Use of intravenous immunoglobulin therapy in autoimmune blistering diseases. *Int Immunopharmacol* 2006;6:557–578.
- 2 Harman KE, Black MM: High-dose intravenous immune globulin for the treatment of autoimmune blistering diseases: an evaluation of its use in 14 cases. *Br J Dermatol* 1999;140:865–874.
- 3 Arredondo J, Chernyavsky AI, Karaoui A, Grando SA: Novel mechanisms of target cell death and survival and of therapeutic action of IVIg in pemphigus. *Am J Pathol* 2005;167:1531–1544.
- 4 Imbach P, Barandun S, d'Apuzzo V, Baumgartner C, Hirt A, Morell A, Rossi E, Schoni M, Vest M, Wagner HP: High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet* 1982;i:1228–1231.
- 5 Lam L, Whitsett CF, McNicholl JM, Hodge TW, Hooper J: Immunologically active proteins in intravenous immunoglobulin. *Lancet* 1993;342:678.
- 6 Bystryń JC, Jiao D, Natow S: Treatment of pemphigus with intravenous immunoglobulin. *J Am Acad Dermatol* 2002;47:358–363.
- 7 Ahmed AR: Intravenous immunoglobulin therapy in the treatment of patients with pemphigus vulgaris unresponsive to conventional immunosuppressive treatment. *J Am Acad Dermatol* 2001;45:679–690.
- 8 Ahmed AR, Sami N: Intravenous immunoglobulin therapy for patients with pemphigus foliaceus unresponsive to conventional therapy. *J Am Acad Dermatol* 2002;46:42–49.
- 9 Sami N, Bhol KC, Ahmed AR: Influence of IVIg therapy on autoantibody titers to desmoglein 1 in patients with pemphigus foliaceus. *Clin Immunol* 2002;105:192–198.
- 10 Sami N, Ali S, Bhol KC, Ahmed AR: Influence of intravenous immunoglobulin therapy on autoantibody titres to BP Ag1 and BP Ag2 in patients with bullous pemphigoid. *J Eur Acad Dermatol Venereol* 2003;17:641–645.
- 11 Bystryń JC, Jiao D: IVIg selectively and rapidly decreases circulating pathogenic autoantibodies in pemphigus vulgaris. *Autoimmunity* 2006;39:601–607.
- 12 Junghans RP: IgG biosynthesis: no 'immunoregulatory feedback'. *Blood* 1997;90:3815–3818.
- 13 Chan PL, Sinclair NR: Regulation of the immune response. V. An analysis of the function of the Fc portion of antibody in suppression of an immune response with respect to interaction with components of the lymphoid system. *Immunology* 1971;21:967–981.
- 14 Li N, Zhao M, Hilario-Vargas J, Prisanh P, Warren S, Diaz LA, Roopenian DC, Liu Z: Complete FcRn dependence for intravenous Ig therapy in autoimmune skin blistering diseases. *J Clin Invest* 2005;115:3440–3450.
- 15 Coutinho A, Kazatchkine MD, Avrameas S: Natural autoantibodies. *Curr Opin Immunol* 1995;7:812–818.
- 16 Avrameas S: Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. *Immunol Today* 1991;12:154–159.
- 17 Kazatchkine MD, Kaveri SV: Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N Engl J Med* 2001;345:747–755.
- 18 Dietrich G, Kazatchkine MD: Normal immunoglobulin G (IgG) for therapeutic use (intravenous Ig) contain antiidiotypic specificities against an immunodominant, disease-associated, cross-reactive idiotype of human anti-thyroglobulin autoantibodies. *J Clin Invest* 1990;85:620–625.
- 19 Rossi F, Kazatchkine MD: Antiidiotypes against autoantibodies in pooled normal human polyclonal Ig. *J Immunol* 1989;143:4104–4109.
- 20 Liblau R, Gajdos P, Bustarret FA, el Habib R, Bach JF, Morel E: Intravenous γ -globulin in myasthenia gravis: interaction with anti-acetylcholine receptor autoantibodies. *J Clin Immunol* 1991;11:128–131.
- 21 Kazatchkine MD, Dietrich G, Hurez V, Ronda N, Bellon B, Rossi F, Kaveri SV: V region-mediated selection of autoreactive repertoires by intravenous immunoglobulin (i.v.Ig). *Immunol Rev* 1994;139:79–107.
- 22 Grando SA, Glukhenky BT, Drannik GN, Kostromin AP, Chernyavsky AI, Barabash TM: Pemphigus and pemphigoid epidermocytes interfere with interleukin cascade reactions. *J Clin Lab Immunol* 1990;32:137–141.

- 23 D'Auria L, Bonifati C, Mussi A, D'Agosto G, De Simone G, Giacalone B, Ferraro C, Ameglio F: Cytokines in the sera of patients with pemphigus vulgaris: interleukin-6 and tumour necrosis factor- α levels are significantly increased as compared to healthy subjects and correlate with disease activity. *Eur Cytokine Netw* 1997;8:383–387.
- 24 Ameglio F, D'Auria L, Cordiali-Fei P, Mussi A, Valenzano L, D'Agosto G, Ferraro C, Bonifati C, Giacalone B: Bullous pemphigoid and pemphigus vulgaris: correlated behaviour of serum VEGF, sE-selectin and TNF- α levels. *J Biol Regul Homeost Agents* 1997;11:148–153.
- 25 Feliciani C, Toto P, Amerio P, Pour SM, Coscione G, Amerio P, Shivji G, Wang B, Sauder DN: In vitro and in vivo expression of interleukin-1 α and tumor necrosis factor- α mRNA in pemphigus vulgaris: interleukin-1 α and tumor necrosis factor- α are involved in acantholysis. *J Invest Dermatol* 2000;114:71–77.
- 26 Andersson UG, Bjork L, Skansen-Saphir U, Andersson JP: Down-regulation of cytokine production and interleukin-2 receptor expression by pooled human IgG. *Immunology* 1993;79:211–216.
- 27 Andersson U, Bjork L, Skansen-Saphir U, Andersson J: Pooled human IgG modulates cytokine production in lymphocytes and monocytes. *Immunol Rev* 1994;139:21–42.
- 28 Skansen-Saphir U, Andersson J, Bjork L, Ekberg C, Fehniger TE, Henter JI, Andersson U: Down-regulation of lymphokine synthesis by intravenous gammaglobulin is dependent upon accessory cells. *Scand J Immunol* 1998;47:229–235.
- 29 Bhol KC, Desai A, Kumari S, Colon JE, Ahmed AR: Pemphigus vulgaris: the role of IL-1 and IL-1 receptor antagonist in pathogenesis and effects of intravenous immunoglobulin on their production. *Clin Immunol* 2001;100:172–180.
- 30 Bayary J, Dasgupta S, Misra N, Ephrem A, van Huyen JP, Delignat S, Hassan G, Caligiuri G, Nicoletti A, Lacroix-Desmazes S, Kazatchkine MD, Kaveri S: Intravenous immunoglobulin in autoimmune disorders: an insight into the immunoregulatory mechanisms. *Int Immunopharmacol* 2006;6:528–534.
- 31 Samuelsson A, Towers TL, Ravetch JV: Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* 2001;291:484–486.
- 32 Bayry J, Lacroix-Desmazes S, Donkova-Petrini V, Carbonneil C, Misra N, Lepelletier Y, Delignat S, Varambally S, Oksenhendler E, Levy Y, Debre M, Kazatchkine MD, Hermine O, Kaveri SV: Natural antibodies sustain differentiation and maturation of human dendritic cells. *PNAS* 2004;101:14210–14215.
- 33 Bayry J, Lacroix-Desmazes S, Carbonneil C, Misra N, Donkova V, Pashov A, Chevailler A, Mouthon L, Weill B, Bruneval P, Kazatchkine MD, Kaveri SV: Inhibition of maturation and function of dendritic cells by intravenous immunoglobulin. *Blood* 2003;101:758–765.
- 34 Paquet P, Kaveri S, Jacob E, Pirson J, Quatresooz P, Pierard GE: Skin immunoglobulin deposition following intravenous immunoglobulin therapy in toxic epidermal necrolysis. *Exp Dermatol* 2006;15:381–386.
- 35 Lawen A: Apoptosis: an introduction. *Bioessays* 2003;25:888–896.
- 36 Majno G, Joris I: Apoptosis, oncosis, and necrosis: an overview of cell death. *Am J Pathol* 1995;146:3–15.
- 37 van Cruchten S, van Den BW: Morphological and biochemical aspects of apoptosis, oncosis and necrosis. *Anat Histol Embryol* 2002;31:214–223.
- 38 Liu X, van Vleet T, Schnellmann RG: The role of calpain in oncotic cell death. *Ann Rev Pharmacol Toxicol* 2004;44:349–370.
- 39 Puviani M, Marconi A, Cozzani E, Pincelli C: Fas ligand in pemphigus sera induces keratinocyte apoptosis through the activation of caspase-8. *J Invest Dermatol* 2003;120:164–167.
- 40 Orlov MD, Chernyavsky AI, Arredondo J, Grando SA: Synergistic actions of pemphigus vulgaris IgG, Fas-ligand and tumor necrosis factor- α during induction of basal cell shrinkage and acantholysis. *Autoimmunity* 2006;39:557–562.
- 41 Frusic-Zlotkin M, Pergamentz R, Michel B, David M, Mimouni D, Bregegere F, Milner Y: The interaction of pemphigus autoimmunoglobulins with epidermal cells: activation of the fas apoptotic pathway and the use of caspase activity for pathogenicity tests of pemphigus patients. *Ann NY Acad Sci* 2005;1050:371–379.
- 42 Alecu M, Alecu S, Coman G, Galatescu E, Ursaciuc C: ICAM-1, ELAM-1, TNF- α and IL-6 in serum and blister liquid of pemphigus vulgaris patients. *Roum Arch Microbiol Immunol* 1999;58:121–130.

- 43 Williamson L, Raess NA, Caldelari R, Zakher A, de Bruin A, Posthaus H, Bolli R, Hunziker T, Suter MM, Muller EJ: Pemphigus vulgaris identifies plakoglobin as key suppressor of c-Myc in the skin. *EMBO J* 2006;25:3298–3309.
- 44 Ekberg C, Nordstrom E, Skansen-Saphir U, Mansouri M, Raqib R, Sundqvist VA, Fernandez C: Human polyspecific immunoglobulin for therapeutic use induces p21/WAF-1 and Bcl-2, which may be responsible for G1 arrest and long-term survival. *Hum Immunol* 2001;62:215–227.
- 45 Grando SA: Autoimmunity to keratinocyte acetylcholine receptors in pemphigus. *Dermatology* 2000;201:290–295.
- 46 Wang X, Breggereg F, Frusic-Zlotkin M, Feinmesser M, Michel B, Milner Y: Possible apoptotic mechanism in epidermal cell acantholysis induced by pemphigus vulgaris autoimmunoglobulins. *Apoptosis* 2004;9:131–143.
- 47 Kawada K, Terasaki PI: Evidence for immunosuppression by high-dose γ -globulin. *Exp Hematol* 1987;15:133–136.
- 48 Prasad NKA, Papoff G, Zeuner A, Bonnin E, Kazatchkine MD, Ruberti G, Kaveri SV: Therapeutic preparations of normal polyspecific IgG (IVIg) induce apoptosis in human lymphocytes and monocytes: a novel mechanism of action of IVIg involving the Fas apoptotic pathway. *J Immunol* 1998;161:3781–3790.
- 49 Sewell WA, Jolles S: Immunomodulatory action of intravenous immunoglobulin. *Immunology* 2002;107:387–393.
- 50 Spahn JD, Leung DYM, Chan MTS, Szeffler SJ, Gelfand EW: Mechanisms of glucocorticoid reduction in asthmatic subjects treated with intravenous immunoglobulin. *J Allergy Clin Immunol* 1999;103:421–426.
- 51 Andersson JP, Andersson UG: Human intravenous immunoglobulin modulates monokine production in vitro. *Immunology* 1990;71:372–376.
- 52 Iwata M, Shimoza T, Tokiwa H, Tsubura E: Antipyretic activity of a human immunoglobulin preparation for intravenous use in an experimental model of fever in rabbits. *Infect Immun* 1987;55:547–554.
- 53 Ichiyama T, Ueno Y, Hasegawa M, Niimi A, Matsubara T, Furukawa S: Intravenous immunoglobulin inhibits NF- κ B activation and affects Fc γ receptor expression in monocytes/macrophages. *Naunyn Schmiedebergs Arch Pharmacol* 2004;369:428–433.
- 54 Stangel M, Schumacher HC, Ruprecht K, Boegner F, Marx P: Immunoglobulins for intravenous use inhibit TNF α cytotoxicity in vitro. *Immunol Invest* 1997;26:569–578.
- 55 Dalakas MC: The role of high-dose immune globulin intravenous in the treatment of dermatomyositis. *Int Immunopharmacol* 2006;6:550–556.
- 56 Viard I, Wehrli P, Bullani R, Schneider P, Holler N, Salomon D, Hunziker T, Saurat JH, Tschopp J, French LE: Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science* 1998;282:490–493.
- 57 Trautmann A, Akdis M, Schmid-Grendelmeier P, Disch R, Brocker EB, Blaser K, Akdis CA: Targeting keratinocyte apoptosis in the treatment of atopic dermatitis and allergic contact dermatitis. *J Allergy Clin Immunol* 2001;108:839–846.

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Skin Involvement in Systemic Autoimmune Diseases

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Abstract

Autoimmune diseases present with varied and broad-ranging cutaneous manifestations. Connective tissue disorders have a plethora of skin manifestations such as rheumatoid nodules in rheumatoid arthritis, psoriatic plaques in psoriatic arthritis, acne and pustulosis in SAPHO syndrome, livedo reticularis and ulceration in antiphospholipid antibody syndrome and xerosis in Sjögren syndrome. Cutaneous manifestations of autoimmune vasculitides such as polyarteritis nodosa, Kawasaki disease, Henoch-Schönlein purpura, cryoglobulinemic vasculitis, Behcet disease, Wegener granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome range from papules, subcutaneous nodules and livedo reticularis, to palpable purpura, hemorrhagic bulla and ulcerating lesions. Pathological skin manifestations in autoimmune endocrinopathies include pretibial myxedema/dermopathy in Graves' disease, diabetic dermopathy and necrobiosis lipoidica in type I autoimmune diabetes mellitus, candidiasis, ectodermal dysplasia, vitiligo and alopecia areata in APECED and uniform hyperpigmentation of the skin in Addison's disease. Autoimmune gastrointestinal disorders such as inflammatory bowel disease (with erythema nodosum), gluten-sensitive enteropathy (with dermatitis herpetiformis), autoimmune hepatitis and primary biliary cirrhosis (with jaundice and pruritus), hematologic/oncologic disorders such as acute and chronic graft-versus-host disease (with skin manifestations ranging from pruritic maculopapular eruptions and lichen planus-like lesions to generalized scleroderma), and paraneoplastic autoimmune dermatoses are discussed as well.

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The skin is a well-known reflection of internal pathologic conditions, and cutaneous autoimmunity as a manifestation of systemic loss of immune tolerance is no exception. Early recognition of subtle skin manifestations would prevent the morbidity and mortality associated with severe internal conditions. This chapter is a review of systemic autoimmune disorders that present with cutaneous manifestations (table 1).

Table 1. Systemic autoimmune diseases with skin involvement

Connective tissue disorders

- Systemic lupus erythematosus¹
- Scleroderma²
- Dermatomyositis³
- Rheumatoid arthritis
- Psoriatic arthritis (psoriasis)⁴
- SAPHO syndrome
- Antiphospholipid antibody syndrome
- Sjögren syndrome

Vasculitis

ANCA negative

- Polyarteritis nodosa
- Kawasaki disease
- Henoch-Schönlein purpura
- Cryoglobulinemic vasculitis
- Behcet disease

ANCA positive

- Wegener granulomatosis
- Microscopic polyangiitis
- Churg-Strauss syndrome

Endocrine disorders

- Graves' disease
- Diabetes type I
- Autoimmune polyendocrine syndrome
- Autoimmune Addison's disease

Gastrointestinal disorders

- Inflammatory bowel disease
- Autoimmune hepatitis
- Primary biliary cirrhosis

Hematologic/oncologic disorders

- Graft-versus-host disease
 - Acute
 - Chronic
- Paraneoplastic autoimmune multiorgan syndrome
- Paraneoplastic dermatoses

¹See chapter by Kuhn and Sontheimer, this vol., pp. 119–140.

²See chapter by Gilliam, this vol., pp. 258–279.

³See chapter by Krathen et al., this vol., pp. 313–332.

⁴See chapter by Nestle, this vol., pp. 65–75.

Mechanisms of Autoimmunity

Thymic negative selection and peripheral tolerance are the two principal mechanisms that normally ensure self-tolerance. In the first process, an autoimmune regulator protein called AIRE has a crucial role in the expression of tissue-specific antigens and in this way directs negative selection of autoreactive T cells [1]. Peripheral tolerance is also a companion mechanism to prevent development of autoimmunity wherein the immune regulation is achieved by specialized regulatory T cells (Tregs). Treg populations that have been identified currently include naturally occurring CD4+CD25+ Tregs which derive from thymus and express the transcription factor Foxp3, CD4+CD25- Tregs with unknown origin which also express Foxp3, and IL-10-secreting Tregs that arise from periphery and acquire CD25 after immune activation [2]. In addition to the different CD4+ Tregs, there are recent reports suggesting that CD8+ T cells also have regulatory function [3]. Tregs prevent the development of autoimmune diseases by limiting the activation of pathogenic autoreactive T cells through dendritic cells. Deficiency of the Tregs or expansion of Treg-resistant pathogenic T cells may result in autoimmune disorders [4].

Connective Tissue Disorders

Connective tissue disorders (CTDs) have a variety of skin manifestations. Systemic lupus erythematosus (SLE) [see chapter by Kuhn and Sontheimer, this vol., pp. 119–140], scleroderma [see chapter by Gilliam, this vol., pp. 258–279] and dermatomyositis [see chapter by Krathen et al., this vol., pp. 313–332] have been discussed comprehensively in other chapters. Other CTDs with skin manifestations are discussed below.

Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a chronic systemic autoimmune disorder characterized by symmetrical synovial inflammation of the peripheral joints and tendon sheets. Extraarticular features include systemic vasculitis, amyloidosis and rheumatoid nodules. Rheumatoid nodules are firm, nontender and usually movable that appear in approximately a quarter of adult patients with RA. These nodules commonly develop within the subcutaneous tissue and on extensor surfaces of forearm, elbow, fingers and knee. The exact etiology of these lesions is unknown. A local vascular trauma is suggested as the initial event leading to continuous presence of extravascular immune complexes and subsequent activation of macrophages. These activated macrophages will then secrete potent cytotoxins and proteinases resulting in fibrinoid necrosis and additional macrophage

recruitment. Minor repeated trauma has been suggested as a contributing factor, since the rheumatoid nodules usually occur in areas prone to trauma [5].

Psoriatic Arthritis

Psoriatic arthritis is a chronic autoimmune disease characterized by inflammatory seronegative arthritis in association with psoriatic skin lesions [see chapter by Nestle, this vol., pp. 65–75]. The disease presents with different clinical expressions which may change from one to another. Currently, the following different patterns are recognized: asymmetric mono- and oligoarticular arthritis, symmetric polyarticular arthritis, distal interphalangeal joint involvement, arthritis mutilans and axial arthritis. Skin lesions include cutaneous psoriatic plaques, dactylitis with diffuse swelling of the entire digit due to tenosynovitis and nail involvement with pitting, ridging and onycholysis [6]. The new criteria developed by the CASPAR study group provide a simplified diagnostic approach with high sensitivity and specificity to delineate psoriatic arthritis from other inflammatory arthropathies.

SAPHO Syndrome

SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis and osteitis) is an uncommon disease that presents with dermatologic and skeletal manifestations. Slightly increased prevalence of HLA-B27 and occasional occurrences of sacroiliitis, inflammatory bowel disease and psoriasis suggest a possible link between SAPHO syndrome and spondyloarthropathies [7].

Antiphospholipid Antibody Syndrome

Antiphospholipid antibody syndrome is characterized by thrombosis, pregnancy complications and typical laboratory abnormalities, including the presence of antiphospholipid antibodies such as anticardiolipin antibodies, anti- β 2 glycoprotein I antibodies and positive lupus anticoagulant test. Any organ may be involved in this syndrome. Cutaneous manifestations are common and precede the thromboembolic events in about a quarter of patients. The most common cutaneous manifestation of antiphospholipid antibody syndrome is livedo reticularis (a violet discoloration of the skin with lattice-like pattern). Other skin lesions include ulcers, digital gangrene, subungual hemorrhages, superficial thrombophlebitis, purpura and necrosis [8].

Sjögren Syndrome

Sjögren syndrome (SS) is an autoimmune condition characterized by dry eyes (xerophthalmia) and dry mouth (xerostomia). It can occur primarily or in the context of other CTDs such as RA and SLE. The involvement of other systemic organs including lung, kidney and nervous system has been reported in

patients with SS. Half of SS patients present with skin manifestations (for example xerosis or pruritus) which are usually nonspecific and less serious than oral, ocular or musculoskeletal symptoms. Palpable purpuric or petechial lesions in the lower extremities are common. Hyperglobulinemic purpura, Raynaud phenomenon, small vessels hypersensitivity vasculitis, urticaria-like lesions, various maculopapular erythematous lesions, cryoglobulinemic vasculitis, subcutaneous nodules, skin ulcers, vitiligo and alopecia have been reported in SS patients [9].

Autoimmune Vasculitis

Polyarteritis Nodosa

Polyarteritis nodosa (PAN) is a segmental necrotizing vasculitis of small and medium-sized arteries. Signs and symptoms of this disease result from diffuse vascular inflammation and ischemia of affected organs. Painful ulcers, livedo reticularis and gangrene are the most common skin manifestations of this disease. PAN is characterized by fibrinoid necrosis of the media, destruction of the internal elastic lamina and leucocytes infiltration followed by substitution of the media by granulation and fibrous tissue as the reparative reaction on histopathology examination. Intimal fibroblastic proliferation, secondary thrombosis, aneurysm formation, and infarction are complications that may occur. Benign cutaneous PAN is mainly limited to medium-sized vessels of the subcutis and not generally accompanied by internal organ involvement [10].

Kawasaki Disease

Kawasaki disease is an acute vasculitic syndrome predominantly affecting children. Mucocutaneous changes, lymphadenopathy and bilateral conjunctivitis are clinical manifestations of the disease. Oral mucosal lesions include erythema of the oropharynx, strawberry tongue, and dry, red and fissured lips. Skin lesions comprise erythema, indurative edema, desquamation of the toes and fingers with palmar erythema, or polymorphous macular exanthema on the trunk. The morbidity and mortality indicate the involvement of coronary, iliac and cerebral arteries. Psoriasiform dermatitis and an erythema multiforme-like interface may be seen on the histopathology. Viruses such as Epstein-Barr virus and bacteria including *Pseudomonas*, *Streptococcus*, *Staphylococcus* and *Rickettsia* have been reported as potential infective triggers [10].

Henoch-Schönlein Purpura

Henoch-Schönlein purpura (HSP) is clinically characterized by purpuric rash, arthralgia or arthritis, abdominal pain, gastrointestinal bleeding and

nephritis. Direct immunofluorescence shows a leukocytoclastic vasculitis (LCV) with perivascular IgA, C3 and fibrin deposits. The etiology and pathogenesis of HSP is not well known. An upper respiratory tract infection usually precedes the disease onset. *Streptococcus* species, *Mycoplasma*, *Bartonella henselae*, parvovirus, human immunodeficiency virus and recently *Helicobacter pylori*, along with food antigens and drugs have been reported as possible triggers. The increased frequency of C4 null alleles and hypocomplementemia among HSP patients proposes a possible role for abnormal complement function and immune complex clearance in the etiopathogenesis of HSP [11, 12].

Cryoglobulinemic Vasculitis

Essential mixed cryoglobulinemia is commonly a complication of chronic hepatitis C; however, high levels of cryoglobulins may also be seen in association with other infections (such as hepatitis B, Epstein-Barr virus, cytomegalovirus, *Treponema pallidum* and *Mycobacterium leprae*, as well as poststreptococcal glomerulonephritis), malignancies (such as multiple myeloma, Waldenström macroglobulinemia, mycosis fungoides and chronic lymphocytic leukemia) and CTDs (such as RA, SLE, SS and HSP). Cryoglobulinemia is classified into three types. Type I (monoclonal immunoglobulin) is usually seen in association with lymphoproliferative diseases. Type II (mixture of polyclonal IgG and monoclonal IgM) and type III (polyclonal IgM) may be seen in association with CTDs and infection. Lower extremity purpura is the most common clinical manifestation which is triggered by cold, trauma, prolonged standing or reaction to a drug or infection. Glomerulonephritis, arthralgia, migratory myalgia, neuropathy and rarely pulmonary vasculitis may also be seen. Type I cryoglobulinemia is characterized by a pauci-inflammatory thrombogenic vasculopathy resulting in infarction. Type II and III cryoglobulinemias are characterized by a necrotizing systemic PAN-like vasculitis. Histomorphology reveals a severe pandermal LCV involving subcutaneous arteries and veins with mononuclear infiltration, fibrin deposition and amorphous eosinophilic precipitates in the lumen of involved vessels [10].

Behçet Disease

Behçet disease is a chronic, recurrent vasculitis characterized by the triad of recurrent aphthous oral ulcers, genital ulcers and ocular lesions. Ninety-eight percent of the patients present with oral ulcerations which are usually the first symptoms. Oral ulcers are round and painful with surrounding erythema and a pseudomembranous covering that commonly occur on the lips, buccal mucosa, gingiva and tongue, and tend to heal spontaneously and without scar. Genital ulcers commonly occur on the scrotum or vulva. They are deep and painful and usually heal with scar. Skin lesions including acneiform lesions, erythema

nodosum, subcutaneous thrombophlebitis, pyoderma gangrenosum-type lesions and palpable purpura are more common on the extensor surface of the legs. Pathergy and dermatographism are seen in Behçet patients [13].

Wegener Granulomatosis

Wegener granulomatosis (WG) is one of the antineutrophil cytoplasmic antibody (ANCA)-associated (commonly c-ANCA) small vessel vasculitides. It is characterized by the involvement of upper respiratory tracts (sinusitis, epistaxis and saddle nose deformity), lower respiratory tracts (cough, hemoptysis and pleuritis), kidney (mild to fulminant glomerulonephritis) and eye. Necrosis, granulomatous inflammation and vasculitis are histopathologic characteristics.

Cutaneous lesions of WG include palpable purpura, pyoderma gangrenosum-like ulcers on the extremities or the head and neck (so-called malignant pyoderma), papules over the extensor joints, oral ulcers, gingival hyperplasia, subcutaneous nodules and acneiform disorders. On light microscopic examination, any of neutrophilic capillaritis, LCV or a PAN-like vasculitis may be seen, usually accompanied by extravascular inflammation and collagen degeneration [10, 14].

Microscopic Polyangiitis

Microscopic polyangiitis (MPA) is another ANCA-associated (mostly p-ANCA) necrotizing vasculitis which involves capillaries, venules and arterioles. Patients have skin involvement and often a focal segmental necrotizing glomerulonephritis. Hemoptysis as a result of a neutrophilic capillaritis is the clinical sign of lung involvement, while oral ulcers, sinusitis and epistaxis indicate nasopharyngeal involvement in MPA patients. Histopathology reveals a severe pandermal LCV. In contrast to WG, here the extravascular inflammation is absent [10, 14].

Churg-Strauss Syndrome

Allergic granulomatosis of Churg-Strauss (AGCS) is the other ANCA-associated (mostly p-ANCA) systemic vasculitis which principally affects smaller blood vessels similar to WG and MPA. AGCS is characterized by allergic rhinitis, asthma and eosinophilia. Drug allergy or allergy shots may be the trigger events for the disease onset. Cutaneous manifestations of AGCS include vasculitic purpura, livedo reticularis or subcutaneous nodules on the scalp or extensor surfaces of the arms. Eosinophilic cardiomyopathy is the most common cause of death in AGCS. In contrast to WG, AGCS commonly involves the heart and gastrointestinal tract, while kidney involvement is rare. Unlike PAN and WG, which do not have vascular immune complex deposits, most AGCS patients show vascular IgE immune complex deposition [10, 14].

Endocrine Disorders

Graves' Disease

Pretibial myxedema or thyroid dermopathy is an uncommon cutaneous manifestation of Graves disease and Hashimoto thyroiditis. Raised waxy lesions in the pretibial area are usually the first sign of thyroid dermopathy. The orange peel (peau d'orange) appearance of the lesions is due to the prominence of hair follicles. Lesions are usually symmetrical and asymptomatic, manifesting in different clinical forms. Diffuse nonpitting edema is the most common form (43%), followed by the plaque form (27%), nodular form (18%) and the most severe form which is associated with elephantiasis (5%). Thyroid dermopathy is almost always associated with relatively severe ophthalmopathy. Typically ophthalmopathy occurs much earlier than dermopathy. Occurrence of pretibial myxedema in unusual sites and high serum concentrations of thyroid-stimulating hormone receptor antibodies are suggestive of a systemic and severe autoimmune process. Localization in the pretibial area indicates the role of mechanical factors and position in the pathogenesis of the lesions. Diagnosis of thyroid dermopathy is generally based on typical pretibial lesions along with a history of Graves' hyperthyroidism and ophthalmopathy; however, sometimes skin biopsy is required to confirm the diagnosis. Histopathology examination of the lesions reveals a large amount of glycosaminoglycans in reticular dermis and massive lymphocyte infiltration [15].

Diabetes Type I

Diabetes type I is an autoimmune condition, often affecting children and young adults, which is characterized by complete lack of insulin hormone and commonly by the presence of circulating antibodies to pancreatic islet cells. Many skin lesions are specific for diabetes mellitus. Diabetic dermopathy or shin spots (very small bounded reddish to brownish lesions on the shins), necrobiosis lipoidica (well-defined waxy plaques with central atrophy and telangiectasia often on the shin), bullosis diabeticorum (irregular tense painless bullae without surrounding inflammation usually on the extremities), sclerodema adultorum (symmetrical waxy indurated skin with occasional erythema commonly on the neck, upper back or the dorsa of the hands and fingers) and granuloma annulare (papules or nodules forming annular shapes on the trunk and the extensor aspect of the extremities) have been associated with the disease. Ischemic ulcers, neuropathic ulcers and lipohypertrophy may also develop as the result of atherosclerosis, neuropathy and local effects of injected insulin, respectively. There are some reports suggesting greater incidence of vitiligo, eczema and psoriasis in patients with type I diabetes [16].

Polyendocrine Syndromes

Autoimmune polyendocrine syndrome type 1 (APS-1) or autoimmune polyendocrinopathy candidiasis ectodermal dysplasia (APECED) is an uncommon hereditary syndrome caused by mutation in a gene coding for a unique transcription factor (AIRE). Recent studies have also shown significant abnormalities in the function of Tregs in human disease, in contrast to the mouse model of the disease. Chronic mucocutaneous candidiasis is the first manifestation of the disease appearing with white thrush on the tongue, candidal esophagitis, nail involvement and even spread of infection to the skin of the hand and face. Hypoparathyroidism and Addison's disease may occur later. The diagnosis is based on presence of at least two of the three conditions (candidiasis, hypoparathyroidism and Addison's disease). Alopecia areata, alopecia totalis, vitiligo, enamel hypoplasia and nail dystrophy can be seen in APECED as the signs of ectodermal dysplasia. Autoimmune thyroid disease, diabetes type I, autoimmune hepatitis and pernicious anemia are other autoimmune disorders which may be seen in association with APECED. APS-2 is genetically more complex than APS-1 and is associated with HLA genes. It is characterized clinically by presence of Addison's disease with either diabetes type I or autoimmune thyroid disease or both. Alopecia and vitiligo also occur in APS-2 but less frequently than in APS-1 [17].

Autoimmune Addison's Disease

Autoimmune Addison's disease (AAD) can occur primarily or in association with other autoimmune diseases. Clinical manifestations of AAD may not appear until the majority of the adrenal cortex is destroyed. The first clinical signs are often general fatigue, weakness, malaise, weight loss, anorexia, nausea and vomiting. Cutaneous manifestations include loss of axillary and pubic hair, dry skin and generalized hyperpigmentation resulting from ACTH excess. Alopecia and vitiligo have been reported in association with AAD [18].

Gastrointestinal Disorders

Inflammatory Bowel Disease

Cutaneous manifestations of inflammatory bowel disease (IBD) include gastrointestinal-cutaneous fistulas (only in Crohn's disease), erythema nodosum (painful red nodules on pretibial area) and pyoderma gangrenosum (papules or pustules leading to deep painful ulcerations with central necrosis). In contrast to erythema nodosum, pyoderma gangrenosum usually occurs in the absence or before the onset of IBD and is not directly correlated with the activity of the underlying IBD. Pyostomatitis vegetans is another uncommon mucocutaneous presentation [19].

Autoimmune Hepatitis

Autoimmune hepatitis is typically a disease of young women. Clinical manifestations include anorexia, fatigue, jaundice, right upper abdominal pain and hepatosplenomegaly [20]. Scleroderma-like lesions, erythema annulare centrifugum, morphea and hypertrichosis lanuginosa acquisita have been reported with autoimmune hepatitis.

Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is generally a disease of middle-aged women with possible autoimmune cause which involves the small intrahepatic bile ducts. Cutaneous manifestations include pruritus, xanthomatous lesions, melanosis and fungal infections (plantar mycoses, onychomycoses and interdigital mycoses) [21]. Keratoconjunctivitis sicca and Raynaud phenomenon are autoimmune diseases that are commonly seen in association with PBC. Systemic scleroderma may also occur in PBC patients [22].

Hematology/Oncology Disorders

Graft-versus-Host Disease

Graft-versus-host disease (GVHD) is an immune syndrome resulting from the reaction of histoincompatible immunocompetent donor cells against the tissues of recipient after transplantation (graft-versus-host reaction).

Acute GVHD

Acute GVHD is a major complication of allogeneic hematopoietic stem cell transplantation, leading to considerable morbidity and mortality. It can occur within days to 3 months after transplantation. The incidence is variable, depending on the degree of HLA matching between donor and recipient, the number of T cells in the graft, the patient's age and the prophylactic regimen.

The pathophysiology of GVHD is explained best as a three-stage process. During the first stage, irradiation and chemotherapy both damage and activate host tissues, leading to the secretion of proinflammatory cytokines such as TNF- α and IL-1 by activated host cells. As the result, expression of adhesion molecules (for example ICAM-1 and VCAM-1) and MHC class II antigens on the host antigen-presenting cells leads to better recognition of host alloantigens by donor T cells. During the second stage, donor T cells proliferate and release Th1 cytokines (IL-2 and IFN- γ), which lead to clonal T cell expansion, stimulation of cytotoxic T cells and natural killer cell reactions and further training of mononuclear phagocytes. In the third stage, a second signal such as

endotoxin (lipopolysaccharide), which may penetrate from damaged intestinal mucosa or skin, prompts the trained mononuclear phagocytes to secrete pathogenic amounts of IL-1 and TNF- α which results in increased local tissue injury [23].

Skin, liver and gut are three major organs commonly involved in acute GVHD. Skin rash is often the first and most common clinical sign. In the early stages of acute cutaneous GVHD, pruritic maculopapular lesions can often be seen on the neck, ears, shoulders, palms and soles which may heal spontaneously or may lead to skin dyspigmentation. In severe GVHD, generalized erythroderma, bullae formation or desquamation can develop, which may even progress to epidermal necrolysis. Drug eruptions and viral exanthema can mimic cutaneous GVHD, clinically making the diagnosis difficult. Histopathologic findings of cutaneous GVHD consist of presence of dyskeratotic epidermal keratinocytes, exocytosed lymphocytes, Langerhans cell depletion, follicular involvement, satellite lymphocytes, vacuolar degeneration of the basal cell layer, intercellular edema, basal cell necrosis, acantholysis and epidermolysis; however, sometimes the histology is not characteristic.

The liver involvement manifests with jaundice, conjugated hyperbilirubinemia and an increased alkaline phosphatase level. Gut involvement is often the most severe and problematic complication of GVHD, manifesting with nausea, vomiting, abdominal cramps, distention, paralytic ileus, intestinal bleeding and diarrhea [24].

Chronic GVHD

Chronic GVHD is still one of the most troublesome delayed complications of allogeneic stem cell transplantation occurring in almost half of patients. Mature donor alloreactive T cells play an important role in both acute and chronic GVHD. In addition to clinical similarities between chronic GVHD and several autoimmune diseases, variable frequencies of autoantibodies such as antinuclear, anti-double-stranded DNA and anti-smooth muscle autoantibodies have been reported in this disease; however, the pathogenetic role of these antibodies is poorly understood [25].

Skin, eyes, oral mucosa, gut, liver and lungs are involved in chronic GVHD. Cutaneous manifestations that are diagnostic for chronic GVHD include poikiloderma, sclerotic lesions, lichen planus-like lesions, morphea-like lesions and lichen sclerosus-like lesions. Other distinctive features include sweat impairment, ichthyosis, keratosis pilaris and hypo- or hyperpigmentation. Erythema, pruritus and maculopapular rash, which commonly present in acute GVHD, may be seen in chronic GVHD as well. Alopecia and nail loss are also common. Lichen-type lesions, hyperkeratotic plaques, xerostomia and ulcers are features of oral involvement in chronic GVHD [26].

Mucocutaneous Paraneoplastic Syndromes

Internal malignancies can be associated with different cutaneous manifestations, known as paraneoplastic syndromes, which may occur before, at the same time, or after cancer development. Moreover, they may be the first sign of tumor recurrence [27]. Several mechanisms have been proposed in the pathogenesis of paraneoplastic syndromes. These include production or reduction of biologically active hormones or growth factors, host immunologic response to tumor antigens that can cross-react with epithelial antigens, autoimmunity induced by dysregulated cytokine production of tumor cells and break down of the barriers between mesenchymal cells and epithelial/neuroectodermal cells by cancer invasion where the released products of these cells could become the targets of cytotoxic killer T cells [28].

Paraneoplastic dermatoses are classified into the following clinicopathologic categories: (1) papulosquamous (epidermal proliferative) disorders, (2) vesiculobullous disorders (3) reactive erythemas, (4) neutrophilic dermatoses, (5) dermal proliferative disorders, (6) dermal deposition disorders and (7) follicular disorders (table 2) [29, 30].

Paraneoplastic Autoimmune Multiorgan Syndrome

Paraneoplastic autoimmune multiorgan syndrome, also known as paraneoplastic pemphigus, is a severe autoimmune inflammatory disease characterized by mucocutaneous eruption (ranging from blisters and erosions to erythema multiforme-like and lichenoid lesions), multiple organ systems involvement (including lung, kidney and muscle), identification of a coexisting malignancy, and immunofluorescence findings. Recent evidence shows that both cell-mediated cytotoxic immunity and production of autoantibodies are involved in the pathogenesis of paraneoplastic autoimmune multiorgan syndrome [31].

Conclusions

Systemic autoimmune diseases manifest varied, often frequent and major pathologic consequences in the skin. Many well-defined systemic autoimmune diseases, such as SLE, scleroderma and dermatomyositis, target the skin as a primary organ in the pathogenic process. Others reviewed in this chapter are more protean. Yet, the skin can be a major target, and patients may suffer significant morbidity as a result of the systemic or organ-specific autoimmune disorder. Astute clinical observation and careful dermatologic inspection provide the critical clues to identifying the underlying autoimmune pathogenesis of the skin disease in many cases. Many biomarkers have been found useful to delineate the primary, underlying systemic autoimmune etiology of these diseases;

Table 2. Paraneoplastic dermatoses

Paraneoplastic syndrome	Clinical presentation
<i>Papulosquamous disorders</i>	
Acanthosis nigricans	Velvety and hyperpigmented papules and plaques often in the flexural areas, neck and dorsum of hand and fingers
Acquired ichthyosis	Diffuse small bran-like to rhomboidal scales on trunk and extremities
Tripe palms	Thickened velvety palms
Leser-Trélat sign	Eruption of widespread seborrheic keratoses, pruritus
Bazex syndrome ¹	Scaly reddish plaques on the nose and ears
Dermatomyositis ¹	Periorbital heliotrope erythema, Gottron papules on finger joints
Vasculitis ¹	Papules, nodules, palpable purpura, ulcers
<i>Vesiculobullous disorders</i>	
Paraneoplastic autoimmune multiorgan syndrome (paraneoplastic pemphigus) ¹	Polymorphous [ranging from lichen planus-like to erythema multiforme to vesicobullous pemphigus-like)] pruritic eruption on trunk and extremities, painful mucosal erosions
Pemphigus vulgaris ¹	Painful dermal and mucosal blisters and ulcers
Pemphigus foliaceus ¹	Fragile superficial dermal blisters and sores
Pemphigoid ¹	Pruritic dermal tense vesicles and bulla
Dermatitis herpetiformis ¹	Intensely pruritic papules and vesicles on elbow, knee and buttocks
Linear IgA dermatosis ¹	Pruritic blistering skin lesions
<i>Reactive erythemas</i>	
Erythema gyratum repens	Concentric erythematous rings with wood-grain appearance migrating rapidly on trunk and proximal parts of extremities
Necrolytic migratory erythema	Annular erythematous macules or papules progressing to blisters with central necrosis on face, trunk, thighs, perianal and oral areas
Erythromelalgia	Burning pain, warmth and redness of the extremities
<i>Neutrophilic dermatoses</i>	
Sweet syndrome ¹	Painful erythematous plaques or nodules on upper extremities
Pyoderma gangrenosum ¹	Deep painful papules, pustules and plaques progressing to enlarged necrotic ulcers
<i>Dermal proliferative disorders</i>	
Multicentric reticulohistiocytosis ¹	Pink to brown granulomatous papules and nodules on hands and face
Necrobiotic xanthogranuloma ¹	Nontender firm dermal or subdermal yellowish, coalescing plaques on periorbital area, trunk and extremities often progressing to ulceration
<i>Dermal deposition diseases</i>	
Scleromyxedema	Firm waxy papules and coalescing plaques symmetrically distributed on hands, face, trunk and extremities
Cutaneous amyloidosis	Waxy papules, plaques and nodules, periorbital purpura
<i>Follicular disorders</i>	
Hypertrichosis lanuginosa acquisita	Soft nonpigmented lanugo-type hair often on the face
¹ Paraneoplastic syndromes with associated autoimmune etiology in selected cases.	

however, the specific pathomechanisms and immune-based testing to explain and use clinically in diagnosis of accompanying skin involvement have been lagging behind. As the immune pathogenesis of these challenging skin diseases is uncovered, better diagnostic and therapeutic interventions will follow. These anticipated findings and therapeutic interventions should provide new insights into more effective treatment of related dermatologic disease limited to the skin as well.

References

- 1 Villaseñor J, Benoist C, Mathis D: AIRE and APECED: molecular insights into an autoimmune disease. *Immunol Rev* 2005;204:156–164.
- 2 O'Garra A, Vieira P: Regulatory T cells and mechanisms of immune system control. *Nat Med* 2004;10:801–805.
- 3 Maile R, Pop SM, Tisch R, Collins EJ, Cairns BA, Frelinger JA: Low-avidity CD8^{lo} T cells induced by incomplete antigen stimulation in vivo regulate naive higher avidity CD8^{hi} T cell responses to the same antigen. *Eur J Immunol* 2006;36:397–410.
- 4 Tang Q, Bluestone JA: Regulatory T-cell physiology and application to treat autoimmunity. *Immunol Rev* 2006;212:217–237.
- 5 Ting PT, Barankin B: Dermacase: rheumatoid nodules. *Can Fam Physician* 2005;51:35, 41, 3.
- 6 Burmester GR, Dörner T, Sieper J: Spondyloarthritis and chronic idiopathic arthropathies; in Rose NR, Mackay IR (eds): *The Autoimmune Diseases*, ed 4. New York, Elsevier, 2006, pp 437–452.
- 7 Kahn MF, Khan MA: The SAPHO syndrome. *Baillieres Clin Rheumatol* 1994;8:333–362.
- 8 Asherson RA, Frances C, Iaccarino L, Kamashta MA, Malacarne F, Piette JC, Tincani A, Doria A: The antiphospholipid antibody syndrome: diagnosis, skin manifestations and current therapy. *Clin Exp Rheumatol* 2006;24(suppl 40):S46–S51.
- 9 Fox RI, Liu AY: Sjogren's syndrome in dermatology. *Clin Dermatol* 2006;24:393–413.
- 10 Crowson AN, Mihm MC Jr, Magro CM: Cutaneous vasculitis: a review. *J Cutan Pathol* 2003;30:161–173.
- 11 Gonzalez-Gay MA, Garcia-Porrúa C, Pujol RM: Clinical approach to cutaneous vasculitis. *Curr Opin Rheumatol* 2005;17:56–61.
- 12 Kim S, Dedeoglu F: Update on pediatric vasculitis. *Curr Opin Pediatr* 2005;17:695–702.
- 13 Bonfilioli AA, Orefice F: Behçet's disease. *Semin Ophthalmol* 2005;20:199–206.
- 14 Gibson LE, Specks U, Homburger H: Clinical utility of ANCA tests for the dermatologist. *Int J Dermatol* 2003;42:859–869.
- 15 Fatourechhi V: Pretibial myxedema: pathophysiology and treatment options. *Am J Clin Dermatol* 2005;6:295–309.
- 16 Bee YM, Ng AC, Goh SY, Tran J, Kek PC, Chua SH, Eng PH: The skin and joint manifestations of diabetes mellitus: superficial clues to deeper issues. *Singapore Med J* 2006;47:111–114; quiz 5.
- 17 Peterson P, Uibo R, Kampe O: Polyendocrine syndromes; in Rose NR, Mackay IR (eds): *The Autoimmune Diseases*, ed 4. New York, Elsevier, 2006, pp 515–526.
- 18 Betterle C, Zanchetta R: Adrenalitis; in Rose NR, Mackay IR (eds): *The Autoimmune Diseases*, ed 4. New York, Elsevier, 2006, pp 501–514.
- 19 Palmon R, Mayer LF: Inflammatory bowel diseases: ulcerative colitis and Crohn's disease; in Rose NR, Mackay IR (eds): *The Autoimmune Diseases*, ed 4. New York, Elsevier, 2006, pp 713–728.
- 20 Mackay IR, Czaja AJ, McFarlane IG, Manns MP: Chronic hepatitis; in Rose NR, Mackay IR (eds): *The Autoimmune Diseases*, ed 4. New York, Elsevier, 2006, pp 729–748.
- 21 Koulentaki M, Ioannidou D, Stefanidou M, Maraki S, Drigiannakis I, Dimoulous P, Melono JM, Tosca A, Kouroumalis EA: Dermatological manifestations in primary biliary cirrhosis patients: a case control study. *Am J Gastroenterol* 2006;101:541–546.

- 22 Selmi C, Coppel RL, Gershwin ME: Primary biliary cirrhosis; in Rose NR, Mackay IR (eds): *The Autoimmune Diseases*, ed 4. New York, Elsevier, 2006, pp 749–666.
- 23 Reddy P: Pathophysiology of acute graft-versus-host disease. *Hematol Oncol* 2003;21:149–161.
- 24 Goker H, Haznedaroglu IC, Chao NJ: Acute graft-vs-host disease: pathobiology and management. *Exp Hematol* 2001;29:259–277.
- 25 Horwitz ME, Sullivan KM: Chronic graft-versus-host disease. *Blood Rev* 2006;20:15–27.
- 26 Baird K, Pavletic SZ: Chronic graft versus host disease. *Curr Opin Hematol* 2006;13:426–435.
- 27 Cohen PR, Kurzrock R: Mucocutaneous paraneoplastic syndromes. *Semin Oncol* 1997;24:334–359.
- 28 Brenner S, Tamir E, Maharshak N, Shapira J: Cutaneous manifestations of internal malignancies. *Clin Dermatol* 2001;19:290–297.
- 29 Chung VQ, Moschella SL, Zembowicz A, Liu V: Clinical and pathologic findings of paraneoplastic dermatoses. *J Am Acad Dermatol* 2006;54:745–762.
- 30 Zappasodi P, Forno C, Corso A, Lazzarino M: Mucocutaneous paraneoplastic syndromes in hematologic malignancies. *Int J Dermatol* 2006;45:14–22.
- 31 Billet SE, Grando SA, Pittelkow MR: Paraneoplastic autoimmune multiorgan syndrome: Review of the literature and support for a cytotoxic role in pathogenesis. *Autoimmunity* 2006;39:617–630.

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Therapeutics and Immune-Mediated Skin Disease

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Abstract

Our increased understanding of the mechanisms of immune-mediated skin disease has led to the growth of therapeutic options for these diseases. Treatment options impact the various levels of the immune response, including the activation and effector function of immune cells. In this review, we give a broad outline of the steps in the immune process that can be altered by immunomodulatory therapy as well as a working model for the potential areas of intervention for new treatments.

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Therapeutic interventions for immunologically mediated skin diseases are as old as recorded history. While man has been aware of the beneficial effects of the sun and tar for skin inflammation for millennia, it has only been in the last few decades that our knowledge of cutaneous immunity has progressed to give even a fundamental understanding of how these therapies work. Additionally, as more intricate information on cutaneous inflammation and immunity becomes available, along with new techniques in genetic engineering, it is possible to develop new therapeutic agents with specificity for particular immune processes. In this chapter, we will outline the basic principles that govern immunotherapy for skin disease and examine how these apply to specific treatment modalities.

Basic Principles of Immunotherapy

The cutaneous immune system is a complex structure. The elements of innate and adaptive immunity that are meant to respond to environmental challenges

are mainly microorganisms. In disease, these same immune responses result in damage to otherwise normal tissue. Thus, while the clinical outcome of normal immunological homeostasis and immunologically mediated disease may be distinct, the basic immune processes are quite similar. There is a fundamental series of processes that leads to the initiation, propagation and effector function of an immune response that results in both health and disease. It is important to note that while it is relatively easy to visualize the processes described below as occurring in a sequential and orderly manner, all of these events happen simultaneously and continuously. The systems described below provide a tool for developing a model for understanding basic immunotherapy, but grossly underestimate the complexity of cutaneous immunity.

The first steps in any immune process are recognition and initial activation. Both the innate and adaptive immune processes require some form of recognition to initiate a response. In innate immunity, this initiation step is usually not specific to the particular bacteria for example, but recognizes large segments of a class of environmental challenges. For example, Toll-like receptor activation of neutrophils and macrophages in response to lipopolysaccharide on Gram-negative bacteria is not specific to any particular species but can initiate a response across many different types of bacteria. The recognition step in adaptive immunity is usually more complex and specific, involving T cell receptors and surface immunoglobulin on B cells identifying specific proteins. The specificity of the adaptive immune response also allows for the development of immunological memory that does not exist in innate immunity.

Activation usually will occur simultaneously with recognition. Activation can be either a simple or complex process, depending upon the cell type and the impetus for activation. Activation of innate processes may only require a single step. Activation of adaptive responses is usually significantly more complicated. T cell activation, for example, requires recognition of the appropriate peptide from an antigen-presenting cell in the context of the proper MHC molecule in addition to a costimulatory signal. Likewise, efficient production of antibody requires T cell help along with ligation of surface antibody on B cells. Importantly, adaptive immune responses generally will require recognition and activation steps. The first step leads to differentiation and amplification as well as to development of immunological memory, while the second step leads to the effective immune response.

Differentiation of immune cells is a process that is generally limited to adaptive immunity. The best example of this process is the differentiation to type I and type II T cells. Depending upon the local environment, T cells will develop a specific cytokine profile. The presence of IL-12 during initial activation will push T cells to develop a type I phenotype and produce IFN- γ . Alternatively, exposure to local IL-4 during activation will direct the cells to

develop a type II profile. This differentiation step is critical in determining the type of immune response elicited and has a significant impact on the types of immunologically mediated disease that will develop.

The steps of recognition, initial activation and differentiation are the foundation for the development of an immune response. However, unless there is a critical mass of inflammatory cells, there will be no clinically apparent response. Amplification is the gathering of potentially active immune cells in the tissue where they can have an impact on local tissue. This amplification process derives from multiple functional systems. The most obvious of these systems is cellular proliferation. Whether in the bone marrow, lymph nodes or local tissues, proliferation of inflammatory cells increases the number available for a response. A more subtle, but equally important mechanism for the amplification of an immune response in local tissue is cellular migration. Soluble factors, such as chemokines, work in conjunction with adhesion molecules on the endothelium to attract immune cells into the local tissues. Additionally, in the skin, keratinocytes may express adhesion molecules that not only attract inflammatory cells to the epidermis but keep them from migrating out.

Finally, after recognition, initial activation and differentiation, and amplification, the immune cells produce their effector response, the response we see in tissue as fighting a pathogen or as an immunologically mediated disease. Importantly, mainly in adaptive responses, effector function may require a secondary activation step. This secondary activation step is best understood with T cells and is superficially very similar to the original activation step. Once activated, immune cells can provide a plethora of effector functions. For example, neutrophils produce toxic oxygen species to induce cellular damage on invading bacteria or infected cells. Macrophages endocytose microorganisms and produce inflammatory cytokines. T helper cells produce cytokines that induce activity in other cell types, while cytotoxic T cells induce apoptosis in target cells and also produce cytokines. Finally, the effector function of B cells is to produce antibody. With any of these elements, it is the effector function of the immune cells that directly fights pathogens or induces the damage to tissue.

The Immune Process and Therapy: A Model for Understanding Immunotherapy

This idealization of the immune process is of central importance in creating a conceptual framework to understand and classify immunotherapy. To identify how an immunotherapy could alter the pathogenic process of a disease,

it is necessary to put that treatment into the context of the immunological response creating that disease. There are five basic methods by which immunological treatment can alter disease according to the outline above: (1) elimination of the immunologically active cells that are responsible for the immune response, (2) blockade of cellular recognition and activation, (3) alteration of differentiation, (4) inhibition of amplification, or (5) reduction in the effector function of cells. While these strategies have had varying levels of success in the design of immunotherapy, all methods of immunomodulation fall within the context of at least one of these five therapeutic principles.

Elimination of the Immunologically Active Cells

Possibly the most obvious method for downregulating a pathogenic immune response is simply removing the immunologically active cells from the tissue. This may occur in a number of ways. Inducing cell death through apoptosis or necrosis can reduce the number of cells present and make any potential ongoing response clinically invisible. For example, massive cell death in neutrophils can rapidly stop a neutrophilic dermatosis. Also, the induction of apoptosis in antibody-producing cells can decrease the activity of bullous disease. Alternatively, the tissue environment may be altered to induce inflammatory cells to migrate out of the diseased tissue. Migration of inflammatory cells out of the tissue, when coupled with an inhibition of influx of new effector cells, can also reduce the likelihood of a sustained immune response. For example, inducing T cells or dendritic cells to migrate out of the skin in psoriasis or atopic dermatitis may induce great clinical improvement.

Blockade of Cellular Recognition and Activation

As mentioned above, these two elements of the immune response are frequently considered together. Recognition by itself may be sufficient for activation with a number of innate immune cells, like neutrophils. However, adaptive responses, dependent upon lymphocyte activity, generally require recognition in association with additional cellular interactions and signaling for the development of an active cell line. The best understood mechanism is the activation steps in T cells. The T cell receptor must bind to a specific peptide in the context of the appropriate major histocompatibility complex (MHC) molecule for recognition to take place. However, activation also requires a second signal, called costimulation, to attain full activation and to prevent anergy and cell death.

The blockade of the recognition and activation steps can be accomplished by a physical interruption of cell-cell signaling. Additionally, the expression of surface molecules, like CD40, CD80 or CD86 can be inhibited. Alternatively, it is thought that some medications, like hydroxychloroquine, may work through

inhibition of antigen processing and presentation, making recognition impossible [1]. Whatever the technique, it is important to remember that blockade of activation can work both on initial and secondary activation in tissue.

Alteration of Differentiation

Changing the differentiation step of the immune process can significantly alter the pathological impact of an immune response. Altering immune differentiation is also termed immune deviation and generally refers to changes in T cell phenotype. Immune deviation is based on the principle that T cells need certain cytokines to differentiate into either type I or type II cells. Once differentiated, cytokines of the alternative phenotype can inhibit the proliferation and activity of the differentiated cell. For example, for a type I, IFN- γ -producing cell, type II cytokines like IL-4 can decrease cellular activity and proliferation. Thus, a treatment based on giving IL-4 could have an impact on the activity of a type I-mediated immune-mediated disease like psoriasis.

Inhibition of Amplification

Amplification relies upon both cellular proliferation and migration into target tissues. Medications that block the production of cytokines and signaling proteins necessary for proliferation can alter this process. Along the same lines, however, medications that block the ability of inflammatory cells to proliferate, such as azathioprine and methotrexate, can also significantly impact immune activity.

Inhibition of specific pathogenic cellular migration into the skin has long been thought of as an outstanding target for the treatment of skin disease. Theoretically, skin-specific homing signals, like expression of cutaneous lymphocyte antigen, could be blocked, limiting disease. More general blockade of cellular migration, for example LFA-1/ICAM-1 (leukocyte function associated antigen-1/intercellular adhesion molecule-1) interactions, would potentially be more potent but could also be more generally immunosuppressive.

Reduction in the Effector Function of the Immune Response

In immune-mediated disease, it is the effector function of inflammatory cells that results in damage to normal tissues. These effector functions vary, including general tissue damage from the secretions of neutrophils, apoptosis induced by cytotoxic T cells, antibody production from B cells, or cytokines secreted by macrophages, dendritic cells and helper T cells. Even after recognition, activation, differentiation and amplification, clinically apparent disease can be reduced or even eliminated by blocking this final step of the pathogenic process.

The most successful strategy for the reduction of effector function is the deactivation of postsecretory cytokines. In particular, agents that block TNF- α , a cytokine produced by many immune cells that is central in a number of inflammatory pathways, have been successful in treating a number of cutaneous diseases. While the best known success for this pathway is in the treatment of psoriasis, other diseases like cutaneous sarcoid have responded to medications that work via this strategy. Thus, given that there are several multiple common pathways that immunologically mediate a multitude of skin diseases, blocking effector function may have a multitude of potential areas of efficacy.

Immunotherapy

The evolution of anti-inflammatory and immunosuppressive drugs progresses from medications that ubiquitously impact almost all elements of the immune process to those that have more specific effects. The hope is that as drugs have more targeted mechanisms, efficacy in the treatment of immunologically mediated disease can be maintained and immune and nonimmune side effects will be reduced. In the last decade, this evolution has involved the advent of biologic immunotherapies that are designed to interact with a single surface or soluble protein and, thus, do not impact other organ systems. A review of a few representative immunotherapies follows to illustrate these principles of immunomodulation.

General Blockade of Immunity: Corticosteroids

The first modern breakthrough in the treatment of immunological disease in general, and in the skin specifically, was the development and use of systemic corticosteroids [2]. The revolutionary impact of corticosteroids, both systemic and topical, on the treatment of immune-mediated skin disease cannot be overstated. We often forget that prior to the widespread use of corticosteroids, diseases like pemphigus vulgaris had significant mortality that is rarely seen today [3]. The great limitation of corticosteroid therapy, however, is the diffuse impact of this class of medication on both the immune system and the body in general.

As one might predict, as the first major class of immunomodulating treatments, corticosteroids have the least specific mechanism and impact all elements of the model. In high doses, corticosteroids induce apoptosis in multiple immunological cell types including eosinophils and lymphocytes [4, 5] and, thus, eliminate effector cells. Corticosteroids can inhibit multiple pathways in the immunological process, including cellular proliferation, cytokine production

and cellular migration. Many of these effects are related to the profound impact of corticosteroids on the transcription factor NF- κ B [6, 7]. Thus, these drugs impact all elements of the immunological model.

Given the multitude of effects of corticosteroids, one would predict that these medications have efficacy in almost all immunologically mediated skin diseases and also have significant immunological side effects. In fact, both of these predictions are true. Corticosteroids, both topical and systemic, are the most commonly prescribed medications for inflammatory skin disease in the world. However, the risks of corticosteroid therapy are great. Recent information on infection in rheumatoid arthritis patients suggests that even very low doses of prednisone (5–10 mg/day) can be of greater risk for the development of systemic infection than methotrexate or anti-TNF therapy [8]. Likewise, since the mechanisms of corticosteroids cross many systems, the well-known side effect profile of this class of drugs should severely limit their use [9].

Other Small Molecules: Antimetabolites

The next series of developments for immunotherapy were other small molecules taken orally, the antimetabolites. These drugs, including methotrexate, cyclophosphamide [10], azathioprine [11, 12] and mycophenolate mofetil [13, 14], work primarily by inhibiting cellular proliferation and partially blocking the amplification step of immunity. Methotrexate, in particular, decreases the number of inflammatory cells in tissue, decreasing cellular migration into tissue as well as decreasing reactivation [15, 16]. These drugs are somewhat specific for a single area in the model and one would expect, correctly, that they do not confer as great a risk for infection as corticosteroids [8]. However, due to the mechanism of these medications, altering basic intracellular processes associated with cell replication and protein synthesis, all of these drugs are limited in dose by multiorgan toxicities.

Targeting the Immune Process: Biologic Immunotherapies

The current trend in immunotherapy is designing medications that target a specific point in the immunological pathway by creating a protein to inhibit the pathway. Using genetic engineering techniques, protein drugs have been developed that specifically target either cell surface proteins on immune cells or soluble cytokines [17]. Specificity is conferred by the use of monoclonal antibodies [17] or naturally occurring cellular receptors [18]. The epitopes that confer specificity are then attached to a molecular backbone, usually related to the Fc portion of human antibody, to create a specific medication that has appropriate pharmacologic properties. The overwhelming predominance in the development and use of these medications for skin disease is in the treatment of psoriasis, but

they have demonstrated efficacy in a variety of other diseases as well. In the next section we will give specific examples of medications that work within the context of the 5 strategies for immunotherapy mentioned above.

Strategy 1 – Elimination of Effector Cells: B and T Cells

Pemphigus vulgaris is an autoimmune blistering disease that is induced by the production of monoclonal antibodies against desmoglein 3. As the exact mechanism by which these antibodies induce epidermal acantholysis is still not fully established, the disruption of the production of these pathogenic antibodies is the primary goal of therapy [19]. Rituximab is an anti-CD20 monoclonal antibody that binds to mature, antibody-producing B cells and has been used for the treatment of B cell lymphoma and autoimmune conditions. Binding of rituximab depletes these cells through a number of potential mechanisms, including drug-induced apoptosis and complement-mediated cytolysis [20–22]. Thus, depletion of potentially antibody-producing cells with rituximab should be effective in the therapy of pemphigus vulgaris.

There has been a number of small series examining the efficacy of rituximab for pemphigus vulgaris [9, 16, 23–27]. These studies consistently demonstrate efficacy in reduction of clinical activity of pemphigus. This clinical improvement correlates with a reduction in circulating anti-desmoglein 3 antibodies and, in some reports, the depletion of CD20+ B cells [24, 25]. Many reports, however, describe significant infectious side effects of treatment, including opportunistic infections and death in a limited number of treated patients, suggesting a need for evaluation of the risk and reward for patients with severe pemphigus vulgaris [26, 27].

One interesting aspect of this strategy is the potential for the induction of longer-term responses. Theoretically, if pathogenic cells are eliminated rather than inhibited, long-term responses may be seen without continued exposure to medication. There have been a number of reports on this effect with rituximab [24, 28]. Further evidence for this mechanism has been derived with treatment experience from the anti-T cell biologic therapy, alefacept, for psoriasis. Alefacept is an LFA-1/Fc fusion protein that binds CD2 on the surface of T cells. This molecule induces depletion of CD45RO+ T cells, a primary effector population in this disease [29–31]. Reduction in T cell numbers has been correlated to the efficacy of this medication [32, 33]. Additionally, patients who respond to alefacept tend to have prolonged responses not seen with other medications for this disease [34]. Thus, while there is a significant need for further investigation, it seems that the depletion of effector immunocytes may provide for longer-term responses to treatment.

Strategy 2 – Blockade of Cellular Activation: Stimulation and Costimulation

The direct blockade of molecules at the site of antigen recognition was the first strategy using biologic immunotherapy for inflammatory skin disease. Anti-CD4 monoclonal antibodies were used to inhibit T cell activity at the site of initial recognition for therapy of psoriasis and atopic dermatitis [35–38]. Additional treatments have been developed to block costimulation, including abatacept and efalizumab. Abatacept is a CTLA4-Ig fusion protein molecule that binds and inactivates the costimulatory function of CD80/86 when binding CD28 on T cells. Absence of this interaction during T cell stimulation can lead to T cell apoptosis or anergy and an incomplete immune response. Abatacept was recently approved for the treatment of rheumatoid arthritis. Early trials of abatacept demonstrated significant clinical improvement as well as reversal of abnormal pathology in psoriatic plaques [39, 40]. Efalizumab will be discussed in greater detail below.

Strategy 3 – Alteration of Cellular Differentiation: Inhibition of Type I Immune Responses in Psoriasis

One of the most intriguing strategies for treating immune-mediated diseases is altering the predominant immunological phenotype. Deviating T cell phenotype and cytokine production patterns should induce improvement in immune-mediated diseases. For example, since psoriasis is traditionally considered a type I T cell-mediated disease, using type II cytokines, like IL-4 or IL-10, should inhibit the disease. Conversely, in a type II disease, giving IFN- γ should be of benefit [41]. In fact, this strategy has shown some limited success. Treatment with IL-4 [42] and IL-10 [43] has demonstrated clinical responses in psoriasis. Alternatively, multiple small studies suggest that treatment with IFN- γ may be beneficial in atopic dermatitis [44–47].

Though this approach has not been pursued commercially, there are reports that suggest this technique could be effective. Treatment of psoriasis with recombinant IL-4 protein has been reported to produce both clinical and histological results. Reports of IFN- γ for atopic dermatitis, however, have had more equivocal results.

One interesting variant on this strategy has recently emerged in the treatment of psoriasis. While the most obvious method of inducing immune deviation would be to add type II cytokines in psoriasis, it may also be possible to inactivate those cytokines that induce a type I response. IL-12 is a critical cytokine in the differentiation of T cells into a type I response. Recently published data

suggest that a monoclonal antibody that inactivates IL-12, CNTO-1275, may have a significant impact on psoriasis [48]. However, since CNTO-1275 also inactivates another cytokine in the IL-12 family, IL-23, it is not certain that immune deviation is its primary mechanism.

Strategy 4 – Inhibition of Amplification: Cellular Migration and Reactivation

Preventing increases in the numbers of effector immune cells in the target tissue can be an effective method for the treatment of immunological disease. As mentioned above, increased numbers of immunocytes can be derived from the blood through migration into the tissue, prevention of cellular migration out of the skin [49] or by increased cellular proliferation locally [50, 51]. Efalizumab is a humanized monoclonal antibody that binds to CD11a, a subunit of LFA-1. LFA-1 on T cells can bind ICAM-1 on endothelium and/or antigen-presenting cells and mediates a central interaction in T cell migration into the skin, maintenance within the skin, and restimulation and expansion of the effector population [52]. Efalizumab has demonstrated efficacy in the treatment of psoriasis [53, 54]. Though the use of efalizumab may have significant effects on T cell activation and expansion, lymphocyte numbers in the circulation increase significantly upon treatment with this medication. This finding suggests that the movement of T cells out of the tissue and into the circulation may be the primary manner in which efalizumab has its effects [55]. The mechanism of action of efalizumab should be applicable to all ICAM-1-dependent T cell-mediated diseases. Recent small reports have suggested that efalizumab may have clinical efficacy in atopic dermatitis as well [56, 57].

Strategy 5 – Inactivation of Effector Function: Anticytokine Therapy

The strategy that has had the greatest impact on the treatment of inflammatory disease is the inactivation of postsecretory cytokines. In particular, the inactivation of TNF- α , a proinflammatory cytokine that is central to the pathophysiology of many diseases, has been successful in the treatment of inflammatory arthritis, inflammatory bowel disease and psoriasis. The treatment of psoriasis with the fusion protein etanercept [58], the chimeric monoclonal antibody infliximab [59] and the human monoclonal antibody adalimumab [60] have been successful. All three drugs have shown high rates of response with the antibody medications having greater efficacy. The mechanism of the effect of TNF inhibition is not fully understood, but may be related to

downstream signaling effects and altering populations of immunoregulatory cells [61–63].

With the recognition of a range of diseases that could possibly be treated with the elimination of a cytokine central to the inflammatory process, numerous case reports and small series have been published to look for nonpsoriatic uses of anti-TNF therapy for cutaneous disease. Importantly, different molecules may have differing effects on certain diseases. For example, sarcoidosis may be treatable with infliximab but is unlikely to respond to standard doses of etanercept [64]. Other diseases for which there are multiple case reports include pyoderma gangrenosum, hidradenitis suppurativa and graft-versus-host disease. Well-defined clinical studies have not been performed on these indications.

Conclusion: The Future of Immunotherapy

While we are in the midst of the biologic era of immunotherapeutics, it is likely that the evolution of immunotherapy will continue. Biologics are limited by their inability to be given orally or, in the case of skin disease, topically. Moreover, their expense in production may limit their use over time. It may be possible, however, to find ways to use the targets defined by biologics to develop new, less expensive, small molecules. Blocking cell-cell interactions with small molecules is a strong possibility. Moreover, small molecules could be found to block very specific intracellular pathways that could limit the activity of immune-mediated diseases. However, the goal of new immunotherapies, either biologic or small molecule, will continue to be to find the most specific pathways to treatment while placing the patient at risk for the fewest adverse outcomes.

References

- 1 Fox RI: Mechanism of action of hydroxychloroquine as an antirheumatic drug. *Semin Arthritis Rheum* 1993;23:82–91.
- 2 Sulzberger MB, Witten VH, Kopf AW: The topical and systemic use of corticosteroids in the treatment of skin disease. *Postgrad Med* 1958;24:379–392.
- 3 Dick SE, Werth VP: Pemphigus: a treatment update. *Autoimmunity* 2006;39:591–599.
- 4 Ohta K, Yamashita N: Apoptosis of eosinophils and lymphocytes in allergic inflammation. *J Allergy Clin Immunol* 1999;104:14–21.
- 5 Scudeletti M, Lanza L, Monaco E, Monetti M, Puppo F, Filaci G, Indiveri F: Immune regulatory properties of corticosteroids: prednisone induces apoptosis of human T lymphocytes following the CD3 down-regulation. *Ann NY Acad Sci* 1999;876:164–179.
- 6 Barnes PJ, Karin M: Nuclear factor- κ B: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997;336:1066–1071.
- 7 Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS Jr: Role of transcriptional activation of IkB α in mediation of immunosuppression by glucocorticoids. *Science* 1995;270:283–286.

- 8 Wolfe F, Caplan L, Michaud K: Treatment for rheumatoid arthritis and the risk of hospitalization for pneumonia: associations with prednisone, disease-modifying antirheumatic drugs, and anti-tumor necrosis factor therapy. *Arthritis Rheum* 2006;54:628–634.
- 9 Dupuy A, Viguier M, Bedane C, Cordoliani F, Blaise S, Aucouturier F, Bonnetblanc JM, Morel P, Dubertret L, Bachelez H: Treatment of refractory pemphigus vulgaris with rituximab (anti-CD20 monoclonal antibody). *Arch Dermatol* 2004;140:91–96.
- 10 Hall AG, Tilby MJ: Mechanisms of action of, and modes of resistance to, alkylating agents used in the treatment of haematological malignancies. *Blood Rev* 1992;6:163–173.
- 11 Loo TL, Luce JK, Sullivan MP, Frei E III: Clinical pharmacologic observations on 6-mercaptopurine and 6-methylthiopurine ribonucleoside. *Clin Pharmacol Ther* 1968;9:180–194.
- 12 Younger IR, Harris DW, Colver GB: Azathioprine in dermatology. *J Am Acad Dermatol* 1991;25:281–286.
- 13 Eugui EM, Mirkovich A, Allison AC: Lymphocyte-selective antiproliferative and immunosuppressive effects of mycophenolic acid in mice. *Scand J Immunol* 1991;33:175–183.
- 14 Eugui EM, Mirkovich A, Allison AC: Lymphocyte-selective antiproliferative and immunosuppressive activity of mycophenolic acid and its morpholinoethyl ester (RS-61443) in rodents. *Transplant Proc* 1991;23:15–18.
- 15 Johnston A, Gudjonsson JE, Sigmundsdottir H, Ludviksson BR, Valdimarsson H: The anti-inflammatory action of methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. *Clin Immunol* 2005;114:154–163.
- 16 Sigmundsdottir H, Johnston A, Gudjonsson JE, Bjarnason B, Valdimarsson H: Methotrexate markedly reduces the expression of vascular E-selectin, cutaneous lymphocyte-associated antigen and the numbers of mononuclear leucocytes in psoriatic skin. *Exp Dermatol* 2004;13:426–434.
- 17 McNiece IK: New cytokines and their clinical application. *Cancer Treat Res* 1999;101:389–405.
- 18 Ryffel B: Safety of human recombinant proteins. *Biomed Environ Sci* 1997;10:65–72.
- 19 Lanza A, Cirillo N, Femiano F, Gombos F: How does acantholysis occur in pemphigus vulgaris: a critical review. *J Cutan Pathol* 2006;33:401–412.
- 20 Smith MR: Rituximab (monoclonal anti-CD20 antibody): mechanisms of action and resistance. *Oncogene* 2003;22:7359–7368.
- 21 Jazirehi AR, Bonavida B: Cellular and molecular signal transduction pathways modulated by rituximab (rituxan, anti-CD20 mAb) in non-Hodgkin's lymphoma: implications in chemosensitization and therapeutic intervention. *Oncogene* 2005;24:2121–2143.
- 22 Pitashny M, Shoenfeld Y: B cell depletion in autoimmune rheumatic diseases. *Autoimmun Rev* 2005;4:436–441.
- 23 Morrison LH: Therapy of refractory pemphigus vulgaris with monoclonal anti-CD20 antibody (rituximab). *J Am Acad Dermatol* 2004;51:817–819.
- 24 Arin MJ, Engert A, Krieg T, Hunzelmann N: Anti-CD20 monoclonal antibody (rituximab) in the treatment of pemphigus. *Br J Dermatol* 2005;153:620–625.
- 25 Ahmed AR, Spigelman Z, Cavacini LA, Posner MR: Treatment of pemphigus vulgaris with rituximab and intravenous immune globulin. *N Engl J Med* 2006;355:1772–1779.
- 26 Schmidt E, Seitz CS, Benoit S, Brocker EB, Goebeler M: Rituximab in autoimmune bullous diseases: mixed responses and adverse effects. *Br J Dermatol* 2007;156:352–356.
- 27 Goh MS, McCormack C, Dinh HV, Welsh B, Foley P, Prince HM: Rituximab in the adjuvant treatment of pemphigus vulgaris: a prospective open-label pilot study in five patients. *Br J Dermatol* 2007.
- 28 Espana A, Fernandez-Galar M, Lloret P, Sanchez-Ibarrola A, Panizo C: Long-term complete remission of severe pemphigus vulgaris with monoclonal anti-CD20 antibody therapy and immunophenotype correlations. *J Am Acad Dermatol* 2004;50:974–976.
- 29 Ellis CN, Krueger GG: Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. *N Engl J Med* 2001;345:248–255.
- 30 Krueger GG, Papp KA, Stough DB, Loven KH, Gulliver WP, Ellis CN: A randomized, double-blind, placebo-controlled phase III study evaluating efficacy and tolerability of 2 courses of alefacept in patients with chronic plaque psoriasis. *J Am Acad Dermatol* 2002;47:821–833.
- 31 Ortonne JP: Clinical response to alefacept: results of a phase 3 study of intramuscular administration of alefacept in patients with chronic plaque psoriasis. *J Eur Acad Dermatol Venerol* 2003;17 (suppl 2):12–16.

- 32 Ortonne JP, Lebwohl M, Em GC: Alefacept-induced decreases in circulating blood lymphocyte counts correlate with clinical response in patients with chronic plaque psoriasis. *Eur J Dermatol* 2003;13:117–123.
- 33 Gordon KB, Vaishnav AK, O’Gorman J, Haney J, Menter A: Treatment of psoriasis with alefacept: correlation of clinical improvement with reductions of memory T-cell counts. *Arch Dermatol* 2003;139:1563–1570.
- 34 Gordon KB, Langley RG: Remittive effects of intramuscular alefacept in psoriasis. *J Drugs Dermatol* 2003;2:624–628.
- 35 Morel P, Revillard JP, Nicolas JF, Wijdenes J, Rizova H, Thivolet J: Anti-CD4 monoclonal antibody therapy in severe psoriasis. *J Autoimmun* 1992;5:465–477.
- 36 Rizova H, Nicolas JF, Morel P, Kanitakis J, Demidem A, Revillard JP, Wijdenes J, Thivolet J, Schmitt D: The effect of anti-CD4 monoclonal antibody treatment on immunopathological changes in psoriatic skin. *J Dermatol Sci* 1994;7:1–13.
- 37 Robinet E, Stamm C, Nicolas JF, Faure M, Mercatello A, Coronel B, Wijdenes J, Bienvenu J, Revillard JP, Claudy A: CD4 monoclonal antibody administration in atopic dermatitis. *J Am Acad Dermatol* 1997;36:582–588.
- 38 Bachelez H, Flageul B, Dubertret L, Fraitag S, Grossman R, Brousse N, Poisson D, Knowles RW, Wacholtz MC, Haverty TP, Chatenoud L, Bach JF: Treatment of recalcitrant plaque psoriasis with a humanized non-depleting antibody to CD4. *J Autoimmun* 1998;11:53–62.
- 39 Abrams JR, Lebwohl MG, Guzzo CA, Jegasothy BV, Goldfarb MT, Goffe BS, Menter A, Lowe NJ, Krueger G, Brown MJ, Weiner RS, Birkhofer MJ, Warner GL, Berry KK, Linsley PS, Krueger JG, Ochs HD, Kelley SL, Kang S: CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *J Clin Invest* 1999;103:1243–1252.
- 40 Abrams JR, Kelley SL, Hayes E, Kikuchi T, Brown MJ, Kang S, Lebwohl MG, Guzzo CA, Jegasothy BV, Linsley PS, Krueger JG: Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. *J Exp Med* 2000;192:681–694.
- 41 Kirschbaum J, Forschner K, Rasche C, Worm M: Modulation of lymphocyte phenotype and function by immunoglobulins. *Br J Dermatol* 2006;154:225–230.
- 42 Ghoreschi K, Thomas P, Breit S, Dugas M, Mailhammer R, van EW, van der ZR, Biedermann T, Prinz J, Mack M, Mrowietz U, Christophers E, Schlondorff D, Plewig G, Sander CA, Rocken M: Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. *Nat Med* 2003;9:40–46.
- 43 Asadullah K, Friedrich M, Hanneken S, Rohrbach C, Audring H, Vergopoulos A, Ebeling M, Docke WD, Volk HD, Sterry W: Effects of systemic interleukin-10 therapy on psoriatic skin lesions: histologic, immunohistologic, and molecular biology findings. *J Invest Dermatol* 2001;116:721–727.
- 44 Hanifin JM, Schneider LC, Leung DY, Ellis CN, Jaffe HS, Izu AE, Bucalo LR, Hirabayashi SE, Tofte SJ, Cantu-Gonzales G: Recombinant interferon γ therapy for atopic dermatitis. *J Am Acad Dermatol* 1993;28:189–197.
- 45 Musial J, Milewski M, Undas A, Kopinski P, Duplaga M, Szczeklik A: Interferon- γ in the treatment of atopic dermatitis: influence on T-cell activation. *Allergy* 1995;50:520–523.
- 46 Schneider LC, Baz Z, Zarcone C, Zurakowski D: Long-term therapy with recombinant interferon- γ (rIFN- γ) for atopic dermatitis. *Ann Allergy Asthma Immunol* 1998;80:263–268.
- 47 Jang IG, Yang JK, Lee HJ, Yi JY, Kim HO, Kim CW, Kim TY: Clinical improvement and immunohistochemical findings in severe atopic dermatitis treated with interferon γ . *J Am Acad Dermatol* 2000;42:1033–1040.
- 48 Krueger GG, Langley RG, Leonardi C, Yeilding N, Guzzo C, Wang Y, Dooley LT, Lebwohl M: A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis. *N Engl J Med* 2007;356:580–592.
- 49 Barker JN, Groves RW, Allen MH, MacDonald DM: Preferential adherence of T lymphocytes and neutrophils to psoriatic epidermis. *Br J Dermatol* 1992;127:205–211.
- 50 Dedrick RL, Bodary S, Garovoy MR: Adhesion molecules as therapeutic targets for autoimmune diseases and transplant rejection. *Expert Opin Biol Ther* 2003;3:85–95.

- 51 Yusuf-Makagiansar H, Anderson ME, Yakovleva TV, Murray JS, Siahaan TJ: Inhibition of LFA-1/ICAM-1 and VLA-4/VCAM-1 as a therapeutic approach to inflammation and autoimmune diseases. *Med Res Rev* 2002;22:146–167.
- 52 Kormeili T, Lowe NJ, Yamauchi PS: Psoriasis: immunopathogenesis and evolving immunomodulators and systemic therapies; U.S. experiences. *Br J Dermatol* 2004;151:3–15.
- 53 Lebwohl M, Tying SK, Hamilton TK, Toth D, Glazer S, Tawfik NH, Walicke P, Dummer W, Wang X, Garovoy MR, Pariser D: A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *N Engl J Med* 2003;349:2004–2013.
- 54 Gordon KB, Papp KA, Hamilton TK, Walicke PA, Dummer W, Li N, Bresnahan BW, Menter A: Efalizumab for patients with moderate to severe plaque psoriasis: a randomized controlled trial. *JAMA* 2003;290:3073–3080.
- 55 Vugmeyster Y, Kikuchi T, Lowes MA, Chamian F, Kagen M, Gilleaudeau P, Lee E, Howell K, Bodary S, Dummer W, Krueger JG: Efalizumab (anti-CD11a)-induced increase in peripheral blood leukocytes in psoriasis patients is preferentially mediated by altered trafficking of memory CD8+ T cells into lesional skin. *Clin Immunol* 2004;113:38–46.
- 56 Takiguchi R, Tofte S, Simpson B, Harper E, Blauvelt A, Hanifin J, Simpson E: Efalizumab for severe atopic dermatitis: a pilot study in adults. *J Am Acad Dermatol* 2007;56:222–227.
- 57 Farshidi A, Sadeghi P: Successful treatment of severe refractory atopic dermatitis with efalizumab. *J Drugs Dermatol* 2006;5:994–998.
- 58 Leonardi CL, Powers JL, Matheson RT, Goffe BS, Zitnik R, Wang A, Gottlieb AB: Etanercept as monotherapy in patients with psoriasis. *N Engl J Med* 2003;349:2014–2022.
- 59 Menter A, Feldman SR, Weinstein GD, Papp K, Evans R, Guzzo C, Li S, Dooley LT, Arnold C, Gottlieb AB: A randomized comparison of continuous vs. intermittent infliximab maintenance regimens over 1 year in the treatment of moderate-to-severe plaque psoriasis. *J Am Acad Dermatol* 2007;56:31–15.
- 60 Gordon KB, Langley RG, Leonardi C, Toth D, Menter MA, Kang S, Heffernan M, Miller B, Hamlin R, Lim L, Zhong J, Hoffman R, Okun MM: Clinical response to adalimumab treatment in patients with moderate to severe psoriasis: double-blind, randomized controlled trial and open-label extension study. *J Am Acad Dermatol* 2006;55:598–606.
- 61 Lizzul PF, Aphale A, Malaviya R, Sun Y, Masud S, Dombrovskiy V, Gottlieb AB: Differential expression of phosphorylated NF- κ B/RelA in normal and psoriatic epidermis and downregulation of NF- κ B in response to treatment with etanercept. *J Invest Dermatol* 2005;124:1275–1283.
- 62 Gottlieb AB, Chamian F, Masud S, Cardinale I, Abello MV, Lowes MA, Chen F, Magliocco M, Krueger JG: TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *J Immunol* 2005;175:2721–2729.
- 63 Gordon KB, Bonish BK, Patel T, Leonardi CL, Nickoloff BJ: The tumour necrosis factor- α inhibitor adalimumab rapidly reverses the decrease in epidermal Langerhans cell density in psoriatic plaques. *Br J Dermatol* 2005;153:945–953.
- 64 Haraoui B: Differentiating the efficacy of the tumor necrosis factor inhibitors. *Semin Arthritis Rheum* 2005;34:7–11.

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